NORTH ATLANTIC TREATY ORGANISATION

RESEARCH AND TECHNOLOGY ORGANISATION

AC/323(HFM-057)TP/196

www.rto.nato.int

RTO TECHNICAL REPORT **TR-HFM-057**

Biotechnologies for Assessment of Toxic Hazards in Operational Environments

(Utilisation des biotechnologies pour l'évaluation des risques toxiques en environnement opérationnel)

Final Report of HFM-057/RTG-009.

Published June 2008

Distribution and Availability on Back Cover

NORTH ATLANTIC TREATY ORGANISATION

RESEARCH AND TECHNOLOGY ORGANISATION

AC/323(HFM-057)TP/196

www.rto.nato.int

RTO TECHNICAL REPORT **TR-HFM-057**

Biotechnologies for Assessment of Toxic Hazards in Operational Environments

(Utilisation des biotechnologies pour l'évaluation des risques toxiques en environnement opérationnel)

Final Report of HFM-057/RTG-009.

The Research and Technology Organisation (RTO) of NATO

RTO is the single focus in NATO for Defence Research and Technology activities. Its mission is to conduct and promote co-operative research and information exchange. The objective is to support the development and effective use of national defence research and technology and to meet the military needs of the Alliance, to maintain a technological lead, and to provide advice to NATO and national decision makers. The RTO performs its mission with the support of an extensive network of national experts. It also ensures effective co-ordination with other NATO bodies involved in R&T activities.

RTO reports both to the Military Committee of NATO and to the Conference of National Armament Directors. It comprises a Research and Technology Board (RTB) as the highest level of national representation and the Research and Technology Agency (RTA), a dedicated staff with its headquarters in Neuilly, near Paris, France. In order to facilitate contacts with the military users and other NATO activities, a small part of the RTA staff is located in NATO Headquarters in Brussels. The Brussels staff also co-ordinates RTO's co-operation with nations in Middle and Eastern Europe, to which RTO attaches particular importance especially as working together in the field of research is one of the more promising areas of co-operation.

The total spectrum of R&T activities is covered by the following 7 bodies:

- AVT Applied Vehicle Technology Panel
- HFM Human Factors and Medicine Panel
- IST Information Systems Technology Panel
- NMSG NATO Modelling and Simulation Group
- SAS System Analysis and Studies Panel
- SCI Systems Concepts and Integration Panel
- SET Sensors and Electronics Technology Panel

These bodies are made up of national representatives as well as generally recognised 'world class' scientists. They also provide a communication link to military users and other NATO bodies. RTO's scientific and technological work is carried out by Technical Teams, created for specific activities and with a specific duration. Such Technical Teams can organise workshops, symposia, field trials, lecture series and training courses. An important function of these Technical Teams is to ensure the continuity of the expert networks.

RTO builds upon earlier co-operation in defence research and technology as set-up under the Advisory Group for Aerospace Research and Development (AGARD) and the Defence Research Group (DRG). AGARD and the DRG share common roots in that they were both established at the initiative of Dr Theodore von Kármán, a leading aerospace scientist, who early on recognised the importance of scientific support for the Allied Armed Forces. RTO is capitalising on these common roots in order to provide the Alliance and the NATO nations with a strong scientific and technological basis that will guarantee a solid base for the future.

The content of this publication has been reproduced
directly from material supplied by RTO or the authors.

Published June 2008

ISBN 978-92-837-0047-0

Table of Contents

	Page
List of Figures	**viii**
List of Tables	**ix**
Explanation of Acronyms, Abbreviations and Nomenclature	**x**
Acknowledgements	**xiii**
Programme Committee	**xiv**
Executive Summary and Synthèse	**ES-1**
Chapter 1 – Terms of Reference and Programme of Work	**1-1**
1.1 Abstract	1-1
1.2 Terms of Reference	1-1
1.2.1 Origin	1-1
1.2.1.1 Background	1-1
1.2.1.2 Justification	1-2
1.2.2 Objectives	1-2
1.2.2.1 Products/Deliverables	1-2
1.3 Programme of Work	1-3
Chapter 2 – Overview of Biotechnologies	**2-1**
2.1 Abstract	2-1
2.2 Scope and Definition of Biotechnology	2-1
2.3 Key Biotechnologies	2-1
2.3.1 Genomics	2-1
2.3.2 Transcriptomics	2-2
2.3.3 Proteomics	2-3
2.3.4 Metabolomics/Metabonomics	2-3
2.3.5 Bioinformatics and Systems Biology	2-3
2.3.6 Biologically-Based Sensors (Biosensors)	2-4
2.4 References	2-4
Chapter 3 – Assessment of Genotoxic Hazards in Operational Environments	**3-1**
3.1 Abstract	3-1
3.2 Introduction	3-1
3.3 Methods for the Assessment of Genotoxic Hazards	3-2
3.3.1 Protein Adducts	3-2
3.3.2 DNA Adducts	3-3
3.3.3 Chromosome Aberrations	3-4
3.3.4 Micronuclei	3-5
3.3.5 Single Cell Gel Electrophoresis (SCGE) – Comet Assay	3-6

3.4 Testing of Environmental Contamination with Genotoxic Agents 3-6
3.4.1 Air Genotoxicity 3-7
3.4.2 Water Genotoxicity 3-7
3.4.3 Soil Genotoxicity 3-8
3.5 References 3-8

Chapter 4 – Monitoring of Air in Confined and Open Spaces 4-1
4.1 References 4-3

Chapter 5 – Toxicology of Mixtures: A Review of Mixtures Assessment 5-1
5.1 Abstract/Resume 5-1
5.2 Executive Summary 5-1
5.3 Introduction 5-3
5.4 Different Approaches to Mixture Assessment 5-4
5.5 Occupational Exposure Assessment 5-5
5.6 Environmental Exposure Assessment 5-7
5.7 Current and Emerging Issues 5-8
5.8 What's on the Horizon? 5-9
5.9 Conclusions 5-9
5.10 Acknowledgments 5-9
5.11 References 5-10

Chapter 6 – Neurobehavioral Toxicity Assessments 6-1
6.1 Abstract 6-1
6.2 Introduction 6-1
6.3 Applications of Neuropsychological Testing 6-2
6.3.1 Health Assessment 6-2
6.3.2 Readiness Status Assessments 6-2
6.3.3 Materiel Safety Evaluations 6-3
6.3.4 Relevance to Military Performance Outcomes 6-3
6.3.5 Performance Assessments 6-3
6.3.6 Warfighter Physiological Status Monitoring Systems 6-4
6.4 Hypotheses Concerning Operational Stressors and Test Performance 6-4
6.4.1 Common Brain Physiology Mechanisms Produced by Military Stressors 6-4
6.4.2 Other Stressors 6-5
6.4.2.1 Chronic Psychological Stress 6-5
6.4.2.2 Head Impact 6-5
6.4.2.3 Sleep Deprivation 6-5
6.4.2.4 Commonalities in Test Outcomes 6-5
6.4.3 Neurotoxic Exposures 6-5
6.4.4 Research Challenges 6-7
6.4.4.1 Lack of Objective Testing for Mood State 6-7
6.4.4.2 More Data Generated than Can be Productively Analyzed 6-7
6.5 Gulf War Illnesses – A Model of Post-Deployment Neuropsychological Concerns 6-7
6.5.1 Post-Deployment Health Concerns 6-7

6.5.2 Neuropsychological Complaints 6-7
6.5.3 Poor Correlation between Neurological Symptom Complaints and Neuropsychological Test Outcomes 6-8
6.5.4 New Studies in Force Health Protection 6-8
6.6 Current and Future Development of the ANAM Tool 6-9
6.6.1 Exploratory Efforts 6-9
6.6.2 The Way Forward 6-9
6.7 Conclusion 6-10
6.8 References 6-10

Chapter 7 – Human Exposure Biomarkers: Permethrin as a Militarily-Relevant Model 7-1
7.1 Abstract 7-1
7.2 Introduction 7-1
7.3 Human Biomonitoring 7-2
7.3.1 Biomarkers of Exposure 7-3
7.3.1.1 Present Applications 7-3
7.3.1.2 Main Prospects 7-4
7.3.1.3 Integrated Access 7-4
7.3.1.4 Quality Assurance 7-5
7.3.1.5 Future Needs 7-5
7.3.2 Military Applications 7-6
7.4 Permethrin 7-6
7.4.1 Physical and Chemical Properties 7-6
7.4.2 Occurrence 7-6
7.4.3 Military Use 7-7
7.4.4 Toxicokinetics 7-8
7.4.5 Health Effects 7-8
7.4.6 Permethrin Biomonitoring of Exposure 7-9
7.4.6.1 Short-Term Markers of Exposure 7-9
7.4.6.2 Longer-term Markers of Exposure 7-10
7.4.6.3 Bundeswehr Approach to Permethrin Biomonitoring of Exposure 7-12
7.5 Conclusion 7-15
7.6 References 7-16

Chapter 8 – Biomarkers of Individual Susceptibility 8-1
8.1 Introduction to Genotypes and Biomarkers of Susceptibility 8-1
8.2 Techniques for Determining Genotypes 8-1
8.3 Classic Examples of Genotyping for Occupational Health 8-3
8.3.1 N-Acetyltransferases (NATs) 8-3
8.3.2 Glutathione S-Transferases (GSTs) 8-3
8.3.3 Glucose-6-Phosphate Dehydrogenase (G6PD) 8-3
8.3.4 Paraoxonase (PON1) 8-4
8.4 The Future of Genotyping in Medicine 8-4
8.5 Epigenetics and Environmental Exposures 8-5
8.6 Potential Genotyping Applications in Military Populations Exposed to Toxic Chemicals 8-5

8.7 References 8-6

Chapter 9 – Neurotoxicological Interactions with Physical and Psychological Stressors **9-1**

9.1 Abstract 9-1
9.2 Introduction 9-1
 9.2.1 The Problem 9-1
 9.2.2 Reviews of Gulf War Illnesses Research 9-2
 9.2.3 Phases of Gulf War Illnesses Research Management 9-3
9.3 Specific Etiologies and Diseases Investigated 9-4
 9.3.1 Diagnostic Criteria for Infectious Diseases – Leishmaniasis 9-4
 9.3.2 Other Infectious Disease Etiologies – Mycoplasma Infection 9-4
 9.3.3 Investigation of Neurotoxicity of Depleted Uranium 9-5
 9.3.4 Ruling Out Teratogenic and Reproductive Effects – DoD Birth Defects Registry 9-5
 9.3.5 Searching for Adverse Effects of Pyridostigmine Bromide 9-6
 9.3.6 Cholinergic Interactions – DEET, Permethrin, and Pyridostigmine Bromide 9-6
 9.3.7 Jet Fuel and other Petroleum Products and Combustion Products of Tent Heaters 9-7
 9.3.8 New Inquiries into Health Consequences of Low Dose Chemical Threat Agents 9-7
 9.3.9 Observed Changes in Immunological Status and Vaccine Associations 9-8
 9.3.10 Neurodegenerative Disease Risks: Amylotropic Lateral Sclerosis (ALS) 9-8
9.4 Chronic Multi-Symptom Disease and Wellness 9-9
 9.4.1 Case Definition of a Poorly Defined Neurological Outcome 9-9
 9.4.2 The Haley Hypothesis 9-10
 9.4.3 A Focus on Chronic Multisymptom Illness 9-10
9.5 Neurotoxin Exposure Treatment Research 9-11
9.6 Force Health Protection Research, 2003+ 9-12
9.7 Conclusions 9-13
9.8 References 9-14

Chapter 10 – Health Risks During the Life Cycle of the Deployed Soldier **10-1**

10.1 Abstract 10-1
10.2 Introduction 10-1
10.3 Recruitment 10-3
10.4 Training Cycles 10-4
10.5 Operational Deployments 10-6
10.6 Post-Military Service 10-8
10.7 Conclusions 10-8
10.8 References 10-11

Chapter 11 – Health Risk Communication: Intended and Unintended Consequences of Biomonitoring **11-1**

11.1 Abstract 11-1
11.2 Scope of Health Risk Communication in Biomonitoring 11-1
11.3 Key Risk Issues, Some Examples 11-2
 11.3.1 The Psychometric Paradigm – Operational Aspects 11-3

11.3.1.1 Key Factors in the Psychometric Paradigm 11-3
11.3.1.2 Motivation – One Brick in the Foundation of Risk Perception and Exposure Resistance 11-4
11.3.1.3 Trust in Commander and Experts – Who do Soldiers Listen to? 11-4
11.3.2 Social Amplification of Risk 11-5
11.3.2.1 Introduction 11-5
11.3.2.2 Toxic Hazards, Stress and Social Amplification of Risks 11-5
11.3.2.3 Media 11-7
11.4 Health Risk Communication and Biomonitoring 11-8
11.5 References 11-10

Chapter 12 – Recommendations 12-1
12.1 The Panel 12-1

Annex A – NATO/PfP Workshop on Environmental and Industrial Health Hazards and Public Health Concerns in International Missions A-1
A.1 Meeting Presentations A-1
A.2 Conclusions A-2
A.2.1 Successful Meeting A-2
A.2.2 Information Sharing A-2
A.2.3 Governing Body A-3

Annex B – 2nd International Workshop on Environmental and Industrial Health Hazards and Public Health Concerns in International Missions B-1
B.1 Meeting Agenda B-1
B.2 Conclusions B-2

List of Figures

Figure		Page
Figure 7-1	Monitoring Chemicals in Occupational and Environmental Sciences	7-2
Figure 7-2	Metabolism of Permethrin	7-12
Figure 7-3	Outdoor Permethrin Fogging Operation During Deployment (Potential Confounding Factor)	7-13
Figure 7-4	Indoor Permethrin Fogging Operation During Deployment (Potential Confounding Factor)	7-14
Figure 9-1	Examples of Some of the Multiple Stressors Surrounding Soldiers During the Gulf War in 1990 – 1991	9-9
Figure 10-1	Predictable Stressors Confront Warfighters During Training and in Deployments	10-2
Figure 10-2	The Role of Research by the Military Operational Medicine Research Program (MOMRP) of the U.S. Army Medical Research and Materiel Command (USAMRMC) in Support of the Center for Health Promotion and Preventive Medicine (CHPPM) for the Health Hazard Assessment (HHA) Process Used by the US Army	10-5
Figure 10-3	Examples of Recent Deployment Hazards Encountered by Peacekeeping Forces	10-7
Figure 10-4	Overview of the U.S. DoD OEH Surveillance Process Including Steps During Pre-Deployment, Initial Deployment, Longer-Term Land-Based Operations, and Post-Deployment Phases	10-9
Figure 10-5	Health Risk Management Scheme Proposed for NATO	10-10
Figure 11-1	There is a Fine Balance in Health Risk Communication Between Providing Accurate Information that Prepares Soldiers Against a Health Risk and Causing Counterproductive Emotional Behaviors	11-2
Figure 11-2	Spectrum of Environmental and Industrial Health Threats	11-4
Figure 11-3	New Enrollments to the Gulf War Health Registry in Relationship to DoD News Releases and Reports between June 1994 and July 1998	11-8

List of Tables

Table		Page
Table 5-1	Links to Relevant Organizations	5-10
Table 6-1	Potential Military Applications of ANAM	6-2
Table 6-2	Examples of Potential Neurotoxicants Relevant to the Military and Occupational and Post-deployment Health Applications	6-6
Table 8-1	Suppliers and Commercially-Available Products for SNP Genotyping	8-2
Table 9-1	Examples of Hypothesized Etiologies of Undiagnosed Symptoms of Gulf War Veterans	9-3
Table 9-2	Some Key Research Accomplishments of the DoD Gulf War Illnesses Research Program	9-11
Table 9-3	Current Force Health Protection (FHP) Research, Showing Examples of Research Initiatives	9-12
Table 9-4	DoD Centers for Deployment Health	9-13

Explanation of Acronyms, Abbreviations and Nomenclature

ACGIH	American Society for Governmental Industrial Hygienists (USA)
ADI	Acceptable Daily Intake
AFPMB	Armed Forces Pest Management Board
ANAM	Automated Neuropsychological Assessment Metric, an experimental computerized system for cognitive function testing developed by the US Army Medical Research and Materiel Command
Apoptosis	Early changes in pathways leading to programmed cell death; used as a marker of accelerated cell death associated with toxic chemical exposures
ATSDR	Agency for Toxic Substances and Disease Registry (USA)
BAT	Biologischer Arbeitsstofftoleranzwert (Biological Tolerance Value for Occupational Exposures)
BDU	Battle Dress Uniform
Br_2CA	cis- 3 -(2,2-dibromovinyl)- 2,2-dimethylcyclopropanol- carboxylic acid (pyrethroid metabolite)
BW	Body Weight
Cl_2CA	see DCCA
C/T	Ratio of Exposure Concentration to TLV
CAS-Nr.	Chemical Abstracts Service Number
CEPA	Canadian Environmental Protection Act (CAN)
CFR	Code of Federal Regulations (USA)
CHPPM	Center for Health Promotion and Preventive Medicine (USA)
CONUS	Continental USA
CPR	Controlled Products Regulations (CAN)
CULTEX	A system for in vivo testing of the effects of toxic gases on lung cells
CW	Chemical Weapons
DCCA	3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (cis or trans) another common abbreviation = Cl_2CA (Pyrethroid metabolite)
DEET	Diethyl toluamide
DFG	Deutsche Forschungsgemeinschaft (German Research Foundation)
DNA	Desoxyribo-Nucleic Acid
DoD	Department of Defense (USA)
EKA	Expositionsequivalente für krebserzeugende Stoffe (Exposure Equivalents for Carcinogenic Substances)
EPI	Exposure/Potency Index
F-PBA	4-fluoro-3-phenoxybenzoic acid (pyrethroid metabolite)
GC-MS	Gas chromatography Mass Spectrometry
GHS	Globally Harmonised Systems

HI	Hazard Index
HPLC	High Performance Liquid Chromatography
HSDB	Hazardous Substances Databank (USA)
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry
IDAA	Individual Dynamic Absorption Application (US Army)
IPCS	International Programme of Chemical Safety
IRIS	Integrated Risk Information Service (USA)
ISS	Integral Search System
JP8	A jet fuel, kerosene-based. It is a replacement for the JP-4 fuel. The U.S. Navy uses a similar formula to JP-8, called JP-5. It was first introduced at NATO bases in 1978. Its NATO code is F-34. It is specified by MIL-DTL-83133 and British Defence Standard 91-87. Commercial aviation uses a similar mixture under the name Jet-A. JP-8 in addition contains icing inhibitor, corrosion inhibitors, lubricants, and antistatic agents.
LC/MSMS	Liquid Chromatography Tandem Mass Spectrometer
LOAEL	Lowest Observed Adverse Effect Level
MIP	Molecular Imprinting
MRL	Minimal Risk Levels
MSDS	Material Safety Data Sheet
NIEHS	National Institute of Environmental Health Sciences (USA)
NIOSH	National Institutes for Occupational Safety and Health (USA)
NOAEL	Non Observed Adverse Effect Level
NOHSC	National Occupational Health and Safety Commission (AUSTRALIA)
NSAID	Non-Steroidal Anti-Inflammatory Drug
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limits
OSHA	Occupational Safety and Health Administration (USA)
PAH	Phenylalanine hydroxylase
PBPK	Physiologically-Based Pharmacokinetic Modelling
PBPK/PD	Physiologically-Based Pharmacokinetic Modelling/Pharmacodynamic
PCR	Polymerase Chain Reaction
PEI	Polyethyleneimine
PEL	Permissible Exposure Limit
Pyrethroids	Broad-spectrum insecticides-- chemicals used to kill a variety of insects. Permethrin is referred to as a synthetic pyrethroid insecticide because, while manmade, it resembles naturally-occuring chemicals with insecticidal properties, called pyrethrins. Pyrethrins are found especially in chrysanthemums.
RAM	Restrictive Access Material
RfD	Reference dose (US EPA)
SCOEL	Scientific Committee on Occupational Exposure Limits (EU)
STEL	Short Term Excursion Limits

TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TDI	Tolerable Daily Intake
TLV	Threshold Limit Value
TTD	Target-organ Toxicity Dose
TWA	Time-weighted Averages
UNECE	United Nations Economic Commission for Europe (UN)
USEPA	Environmental Protection Agency (USA)
WHMIS	Workplace Hazardous Materials Information System
WHO	World Health Organisation
WOE	Weight of Evidence Modification

Acknowledgements

The working group wishes to acknowledge the foresight and contribution of Dr. Henk Benschop to the concept of a focus on biomonitoring against occupational and environmental chemical exposures that builds on the experience of the chemical defense community.

In addition, we gratefully acknowledge the hosts of the HFM-057/RTG-009 committee meetings that occurred in each of these locations:

- Paris, France
- Fort Detrick, Frederick, Maryland, USA
- Schloss Orienstein, Dietz, Germany
- TNO Prins Maurits, Rijswijk, Netherlands
- Toronto, Canada
- NBC-skydd, FOI, Umea, Sweden
- Leopold’s Kazerne, Gent, Belgium
- Natick, Massachusetts, USA

Programme Committee

[National representatives; authors on individual chapters may be more extensive]

BEL: Lieutenant Colonel Christian Carton, MD; Colonel Cor Belanger, MD

CAN: Major Yvonne Severs

CZE: Colonel Assoc Prof Jiri Kassa, MD, PhD

DEU: Colonel Dr. Klaus-Gerhard Mross; Lieutenant Colonel Dr. Jeannot Zimmer

NLD: Agnes A. Tan, PhD; Marijke Mol, PhD; Marijke W. Valstar, Pharm., MPH; Major Herman Steenbergen; Jan Langenberg, PhD, Pharm., (Chair, until 2005); Henk Benschop, PhD (Chair, until 2004)

SWE: Gudrun Cassel, PhD; Birgitta Liljedahl, Senior Research Officer; Lieutenant Colonel Claes Ivgren, Vet Surg

USA: Paul Knechtges, PhD (Chair, from 2005); Colonel Karl Friedl, PhD (recorder/report editor); Richard Stotts, DVM, PhD; David Lam MD, MPH (assistant report editor)

Biotechnologies for Assessment of Toxic Hazards in Operational Environments

(RTO-TR-HFM-057)

Executive Summary

This group focused on markers of exposure for assessment of neurotoxicological threats from non-threat agents. Starting with reviews of standard approaches to toxic industrial chemicals and toxic industrial materials (TIC/TIMs), this group considered the specialized health risks to deployed military forces arising from exposure to toxic hazards chemicals that usually involve mixtures and interactions with other stressors and conditions. To narrow the discussion, two model systems were evaluated in detail, permethrin and JP8. These compounds represent militarily relevant chemical mixtures that are inhalation and dermal exposure hazards with neurotoxicological potential.

Participating countries had various contributions to new research, evaluation, and discussion of approaches to assessing health and performance risks of these two categories of chemicals, ranging from neurobehavioral to special in vitro exposure test systems and cellular biomarkers. Interactions with physical factors (e.g., heat, dust, work/exercise), psychological stress, and other chemical exposures were evaluated. Communicating health risks to military forces to improve protective measures is itself a potential health risk and requires additional specialized research to establish rules of communication to achieve optimal compliance with safety and protective measures and minimal reductions. Two international Environmental and Industrial Health Hazard (EIHH) workshops paralleled the efforts of this panel and expanded contributions to this work.

Further work in these areas is being conducted with agreements to continue sharing of information on different approaches to assessing neurotoxicological risks. Recommendations were made for NATO and national implementation involving further development of efficient processes for early predeployment consideration of potential threats, assessment and monitoring of neurochemical hazards, and lifecycle health monitoring of exposed individuals.

Utilisation des biotechnologies pour l'évaluation des risques toxiques en environnement opérationnel

(RTO-TR-HFM-057)

Synthèse

Ce groupe avait pour thème les marqueurs d'exposition pour l'évaluation des menaces neurotoxiques dues à des agents non hostiles. A partir de revues des approches standard relatives aux produits chimiques industriels toxiques et aux matières industrielles toxiques (TIC/TIM), ce groupe a examiné les risques sanitaires spécifiques pour les forces militaires en déploiement dus à une exposition aux risques toxiques par produits chimiques en tenant compte des mélanges mais aussi de l'influence de facteurs liés au stress ou conditions particulières. Afin de resserrer la discussion, deux systèmes de modèles ont été évalués en détails, perméthrine et JP8. Ces composés représentent des mélanges chimiques susceptibles d'emploi en milieu militaire et présentant des risques d'exposition par inhalation et contact dermique avec un potentiel neurotoxique.

Les pays participants ont apporté des contributions diverses à une nouvelle recherche, évaluation et discussion des méthodes permettant d'évaluer les risques pour la santé et les performances de ces deux catégories de produits chimiques, allant de systèmes de test neurologique aux expositions spéciales in vitro et aux marqueurs cellulaires. Les interactions avec les facteurs physiques (par exemple, chaleur, poussière, travail/exercice), le stress psychologique et d'autres expositions chimiques ont été évaluées. La communication des risques sanitaires aux forces militaires en vue d'améliorer les mesures de protection constitue en elle-même un risque sanitaire potentiel et demande une recherche spécialisée complémentaire afin d'établir des règles de communication permettant d'assurer une conformité optimale aux mesures de sécurité et de protection et un minimum de réductions. Deux ateliers internationaux sur les risques sanitaires environnementaux et industriels (EIHH) ont eu lieu en parallèle de cette commission et ont étendu les contributions à ce travail.

D'autres travaux dans ces domaines sont en cours avec des accords de partage continu des informations sur les différentes méthodes d'évaluation des risques neurotoxiques. Des recommandations ont été émises pour la mise en place aux niveaux national et OTAN d'un développement complémentaire de procédés efficaces permettant une prise en compte précoce des menaces potentielles avant déploiement, l'évaluation et le contrôle des risques neurochimiques, et le contrôle sanitaire en cours de vie des individus exposés.

Chapter 1 – TERMS OF REFERENCE AND PROGRAMME OF WORK

by

M.S. Nieuwenhuizen and J.P. Langenberg, Ph.D.
TNO Defence, Security and Safety BU4 Biological and Chemical Protection
Rijswijk, Netherlands

1.1 ABSTRACT

The activities of the HFM-057/RTG-009 on research and development in the area of biotechnology as applied to the assessment of toxic hazards in operational environments are outlined. These environments are increasingly dangerous from a toxic hazards point of view while at the same time societal acceptance levels are increasingly becoming lower. The activities of the first term ("protection against adverse effects of toxic hazards") and the new tasks for the second term ("biotechnologies for assessment of toxic hazards in operational environments") of the working group are summarized.

1.2 TERMS OF REFERENCE

1.2.1 Origin

1.2.1.1 Background

Public opinion in today's society demands an increasing level of safety and health care for military personnel under operational and peacetime conditions, not restricted to the traditional military threats such as battle injury and infectious diseases. Potential harm from toxic exposure has been recognised increasingly as a threat, in Peacekeeping and Peace Enforcement operations, as well as in classical combat. Troops may be exposed to harmful chemicals as a result of; inadequate environmental protection in the area of operations; industrial accidents; sabotage; or the intentional or unintentional actions of enemy or friendly forces.

Traditionally, the medical protection of soldiers against non-battle injuries has been concentrated on the prevention of infectious diseases. Consequently, risk assessment of non-weaponized toxic hazards in operational areas and on the battlefield has been relatively neglected. Pertinent toxicological research should involve sources and levels of exposure, and the consequent effects thereof on the military. Eventually, this research should better protect health and ensure mission performance of military personnel through improved risk management. Furthermore, the risk of post-deployment illness and disability, resulting from exposures during deployment, will be reduced.

Given these concerns, it is recommended that an explicit effort to identify, quantify and minimize the effects of toxic exposures, especially those which are characteristic for the military environment should be pursued. Such an effort could serve as a venue for initiation and effective co-ordination of military-toxicological research by experts.

In recent years new (bio)technologies have emerged that may be relevant to address the problems mentioned above. The added value of these biotechnologies should therefore be explored.

1.2.1.2 Justification

An increased level of safety and health care for military personnel under operational conditions will improve combat readiness and effectiveness, and therefore the probability of successful mission completion. An additional (non-operational) benefit is that risk of post-deployment illness and disability, resulting from exposures during deployment, will be reduced.

1.2.2 Objectives

HFM-057/RTG-009 will evaluate new and emerging biotechnologies to assess toxic hazards and exposure under operational conditions. This evaluation should lead to identification of areas in which coordinated research efforts are required.

The specific goal of HFM-057/RTG-009 is to facilitate the communication and coordination of environmental and occupational health research and toxicological research among the participating countries. This goal will be realized through regular meetings of the Task Group. More specifically, the Task Group will:

- Evaluate the potential benefits of emerging biotechnologies to protect health and mission performance;
- Provide input to improve guidelines for health and mission performance risk assessment of military-relevant compounds in the operational environment;
- Identify knowledge gaps in inhalation toxicology of military-relevant compounds;
- Identify knowledge gaps in neurobehavioral toxicology of military-relevant compounds and other environmental stressors;
- Identify and propose the development of (specific) biomarkers for diagnostic and prognostic purposes;
- Promote the development of biomarkers of susceptibility, such as polymorphisms;
- Promote investigation of the interactive effects of physical (e.g. heat) and psychological stress on the toxicity of military-relevant compounds;
- Evaluate new methods for studying the toxic hazards of exposure to military-relevant mixtures or combinations of exposures to multiple stressors;
- Consider technologies (e.g. molecular epidemiology) for assessment of health risks through the life cycle of military personnel;
- Identify the unique risks of exposure to military-relevant compounds in confined spaces;
- Promote the importance of health risk communications research; and
- Increase awareness of meetings relevant for the Task Group.

1.2.2.1 Products/Deliverables

- The major product will be a technical report on biotechnologies for assessment of toxic hazards in the operational environment.
- The Task Group will publish an inventory and critical evaluation of existing databases for biomarkers for prognostics and diagnostics and field relevant assessment methods.

- At least one workshop on biotechnologies will be organized and conducted by the Task Group.
- The Task Group will publish the key findings of the Task Group in relevant open literature journals.

HFM-057/RTG-009 is authorized to conduct its actions under these Terms of Reference from January 2004 until December 2006.

1) RESOURCES

A) Membership

Military environmental and occupational health research is conducted in participating NATO and PfP countries. Representation in HFM-057/RTG-009 by participating countries should be by environmental and occupational health researchers, toxicologists, physicians, neuroscientists, biochemists, chemists, biologists, and other scientific researchers having experience in the toxicology of relevant compounds and/or subjects.

B) National and/or NATO resources needed

Special needs of the Task Group should be limited to the use of appropriate secure facilities for the conduct of executive and scientific meetings.

C) RTA resources needed

None foreseen.

II) SECURITY CLASSIFICATION LEVEL

Information exchanges between members of the Task Group may be conducted up to and including the NATO RESTRICTED level.

III) PARTICIPATION BY PARTNER NATIONS

In principle, the Task Group will be open to participation of Partnership-for-Peace (PfP), Mediterranean Dialogue Initiative (MDI), and other nations.

IV) LIAISON

Liaison by members of the Task Group may be conducted with the NBC Medical Working Party, with Task Group 4 on Prophylaxis and Therapy against Chemical Agents, and with other relevant RTA study groups regarding work on environmental sampling/analysis and biomonitoring, especially the group on sampling and identification.

1.3 PROGRAMME OF WORK

The potential benefits to the protection of health and mission performance of emerging biotechnologies will be evaluated by the Task Group.

The Task Group will provide input to improve guidelines for health and mission performance risk assessment of military-relevant compounds in the operational environment.

Knowledge gaps in inhalation toxicology of military-relevant compounds will be identified.

Knowledge gaps in neurobehavioral toxicology of military-relevant compounds and other environmental stressors will be identified.

The Task Group will identify and propose the development of specific biomarkers for diagnostic and prognostic purposes.

The Task Group will promote the development of biomarkers of susceptibility, such as polymorphisms.

The Task Group will promote investigation of the interactive effects of physical (e.g. heat) and psychological stress on the toxicity of military-relevant compounds.

New methods for studying the toxic hazards of exposure to military-relevant mixtures or combinations of exposures to multiple stressors will be evaluated.

Technologies (e.g. molecular epidemiology) for assessment of health risks through the life cycle of military personnel will be considered.

The unique risks of exposure to military-relevant compounds in confined spaces will be identified.

The Task Group will promote the importance of health risk communication research.

In order to fulfil the Programme of Work, the Task Group will meet twice a year in the various participating nations.

Deliverables – The Task Group will produce:

- A comprehensive technical report on biotechnologies for assessment of toxic hazards in the operational environment. This is the major deliverable of the Task Group.
- An inventory and critical evaluation of existing databases for biomarkers for prognostics and diagnostics and field relevant assessment methods.
- At least one workshop on biotechnologies.
- Publication(s) of the key findings of the Task Group in relevant open literature journals.
- Technical reports on specific subjects as considered opportune.

(This TOR and POW were written at the start of the current committee effort on March 19, 2004).

Chapter 2 – OVERVIEW OF BIOTECHNOLOGIES

by

Paul L. Knechtges, Ph.D.
U.S. Army Center for Environmental Health Research, Fort Detrick, MD, U.S.A.

2.1 ABSTRACT

Relevant "–omics", informatics, and biosensor technologies are defined and briefly characterized in a summary of key tools of importance to laboratory investigations of toxic hazards.

2.2 SCOPE AND DEFINITION OF BIOTECHNOLOGY

The term biotechnology is used to describe practical applications of the life sciences, ranging from medicine and agriculture to bio-inspired materials. Although biotechnology has been around for centuries (e.g. wine and medicines), the biotechnology industry grew very rapidly during the 1990s. Part of the reason for this rapid growth was the integration of the life sciences with other enabling technologies such as computers and analytical chemistry by the pharmaceutical manufacturing industry. With the announcement in 1999 that the human genome had been sequenced, a new era of biotechnology known as "genomics" was ushered into research and development.

Given the broad scope of biotechnology, it is very difficult to define it in simple and inclusive terms. Nevertheless, under the auspices of the U.S. National Research Council, the Army Board on Science and Technology published a report that defined biotechnology as a technology with one or both of the following characteristics:

- It uses organisms, or tissues, cell, or molecular components derived from living things, to act on living things.
- It acts by intervening in the workings of cells or the molecular components of cells, including their genetic material [1].

A great number of applications are encompassed by this definition. For the purposes of this Task Group's report, the following section focuses on those biotechnologies judged to be most relevant in protecting NATO troops from toxic hazards during deployments.

2.3 KEY BIOTECHNOLOGIES

2.3.1 Genomics

The term genomics was coined to describe the scientific discipline interested in information about the entire complement of a cell's DNA, referred to as the genome. Prior to the era of genomics, DNA was sequenced on a relatively small scale, usually gene by gene. However, with the advent of modern sequencers and computer technology, institutions have undertaken the sequencing of the entire genomes of many types of organisms. As of January 2005, an estimated 1,251 genome sequencing projects were identified, with 250 genomes completely sequenced and published [2].

The widespread availability of DNA sequences in genomic databases led to additional terms such as ***functional genomics***, which refers to the study of gene functions. Other terms like transcriptomics, proteomics, and metabonomics/metabolomics have also emerged. These "-omics" technologies are described below.

The sequencing of human and animal genomes has permitted comparative study of DNA sequences using super computers and advanced computational algorithms. The results have revealed much about the similarity (i.e. homologies) of coding regions in the DNA (exons) between the different genomes and the total number of possible genes. For example, the genome of the simple nematode *Caenorhabditis elegans* has over 83% of its protein-encoded DNA conserved in the human genome [3]. Higher levels of homology to the human genome are observed with vertebrates, particularly mammals.

By using DNA sequence data in human genome databases, researchers are identifying sequence variations between groups of individuals, which may eventually help explain differences in resistance or susceptibility to genetic and environmentally-induced diseases. A particular emphasis has been placed on mapping variations, known as single nucleotide polymorphisms (SNPs) for use as genetic markers. Over 10 million SNPs are thought to be present in the approximately 3 billion sequences of the human genome. Many SNPs are inherited together in "blocks" that are called haplotypes; the number of haplotypes in the human genome is estimated at 300,000 or more. Compared with SNPs, haplotypes represent a more tractable number of genetic markers for identifying genes related to disease susceptibility or resistance. The human haplotype map was released in 2003 and is now publicly available [4, 5].

One of the greatest leaps forward in biotechnology is the development of gene microarrays, which permit highly multiplexed analysis of genes and gene transcripts (see Annex A and the next section). Several microarrays are already commercially available that can simultaneously determine over 100,000 SNPs in an individual. More densely packed SNP microarrays, and other technologies for measuring genetic variation among individuals, are likely to become available in the near future. These technologies represent unprecedented power to study human genetic variation, which could help identify individual resistance and susceptibility to diseases and responsiveness to medical treatment options.

2.3.2 Transcriptomics

The term ***transcriptomics*** refers to the global measurement and study of messenger RNA (mRNA) that has been transcribed from a cell's DNA. These pieces of mRNA can be translated into peptides or proteins, or used as guides for the synthesis of proteins by ribosomes. Other types of RNA perform other functions such as gene regulation and enzymatic activity. The amount of various mRNAs in the cell is indicative of its cell type and physiological state. The two most important biotechnologies which have enabled the discipline of transrciptomics are microarrays and real-time polymerase chain reaction (RT-PCR).

Microarrays for transcriptomics research are designed with either complementary DNA (cDNA) or oligonucleotide probes that hybridize with mRNA from cells. A variety of methods are used to attach these probes to the microarray, and the number of probes incorporated in a single microarray can number in the tens of thousands. The commercially-available human genome microarray, for example, can detect over 30,000 known transcripts [6]. The transcription products are fluorescently labelled, and a scanner is used to convert light intensities for each probe spot into numeric values for data analysis.

The great number of probes on a microarray requires specialized data analysis techniques, because even a fraction of one percent experimental error can result in thousands of false positives or negatives. Researchers

have published various methods for reducing the likelihood of a false positives or negatives, and new computational approaches are continually being developed. In addition, computer software is commercially available that can perform many of the calculations and data manipulation functions needed for microarray data analysis.

2.3.3 Proteomics

The term ***proteomics*** refers to the global or comprehensive study and analysis of an organism's proteins (its proteome). The proteins of an organism catalyze enzymatic reactions, provide cell structures, serve as cellular signals, and provide many other cellular and inter-cellular functions. The complexity of the proteome greatly exceeds that of the genome. More than 130 different covalent modifications of proteins are known and variable RNA processing events such as alternative splicing and termination increase the number of possible proteins expressed from a single gene.

Advances in proteomics have been driven by increasingly sensitive analytical instrumentation, particularly mass spectrometry, and by powerful computational methods. Microarrays are also emerging for proteomics applications, but they have not yet matched the practicality of genomic and transcriptomic microarray technology. Nevertheless, many biomedical industry analysts predict that proteomics will yield more practical applications such as drug targets and biomarkers. Consequently, ongoing research and development is aimed at increasing the throughput of proteomics methods.

2.3.4 Metabolomics/Metabonomics

Technologies such as nuclear magnetic resonance (NMR) and mass spectrometry (MS) permit the profiling of an entire set of cellular metabolites (the metabolome); this area of investigation is known as ***metabolomics***. The term ***metabonomics*** refers to the study of the complement of small molecule metabolites that change in response to some challenge. Since metabolites are the products and by-products of many biosynthetic and catabolic pathways, the applications of metabolomics include disease diagnosis and the identification of drugs or chemical exposures.

2.3.5 Bioinformatics and Systems Biology

The burgeoning growth of sequence data from genomics, transcriptomics, and proteomics has necessitated development of a new discipline called ***bioinformatics***. This discipline manages and analyzes biological data, including microarray data analysis, protein structure determination, hypothesis generation, and other data-intensive biological research. Bioinformaticists must be knowledgeable of biology as well as computer and software technologies. New and future generations of biologists are being trained to routinely use bioinformatic tools along with advanced laboratory equipment to take full advantage of advances in biotechnology.

Systems biology encompasses analyzing and modelling biological systems ranging from genes, proteins, or metabolites to an overall model of a disease state. The approach in systems biology is iterative and involves interaction between computer simulations (*in silico*) with actual *in vivo* and/or *in vitro* experiments. The results are increasingly accurate models of biological processes over time that can be used to make predictions of responses to therapeutic agents, environmental exposures, or other perturbations to a biological system. Both bioinformatics and analytical technologies such as microarrays are essential to systems biology and to the modelling of complex biomolecular networks.

2.3.6 Biologically-Based Sensors (Biosensors)

Biosensors consist of a biological component (biomolecule, cell, or tissue) and a signal transducer. Over the past decade, technologies have emerged that permit the arrangement of specific biomolecules on a microchip and the culture of cells and tissues within microelectrical mechanical systems (MEMS). These biological components can act as sensors when coupled with transducers that convert biological responses into electrical signals or other types of signals that can be measured (e.g. optical, vibration, acoustic). The culture of cells and tissues in a very small package has many advantages over relatively cumbersome *in vitro* methods found in laboratories. Uses for these biosensors include high throughput screening of pharmaceuticals and the possibility of field-portable sensors for chemical hazards or pathogens.

2.4 REFERENCES

[1] Committee on Opportunities in Biotechnology for Future Army Applications, Board on Army Science and Technology, Division on Engineering and Physical Sciences, National Research Council. *Opportunities in Biotechnology for Future Army Applications*. Washington, D.C.: National Academy Press, 2001.

[2] www.genomesonline.org – (Last accessed 31 January 2007).

[3] Lai, C. et al. Identification of novel human genes evolutionarily conserved in caenorhabditis elegans by comparative proteomics. Genome Res 2000; 10(5): 703-713.

[4] International HapMap Consortium. The international HapMap project. Nature 2003; 426(18/25):789-796.

[5] www.hapmap.org – (Last accessed 31 January 2007).

[6] Addonizio, M. The great chip chase. Scientist 2003; 17(17):49.

Chapter 3 – ASSESSMENT OF GENOTOXIC HAZARDS IN OPERATIONAL ENVIRONMENTS

by

R. Stetina and J. Kassa
Department of Toxicology, Faculty of Military Health Sciences
Hradec Kralove, Czech Republic

3.1 ABSTRACT

Key assay methods for detection and identification of genotoxic hazards are outlined and appropriate applications are discussed.

3.2 INTRODUCTION

Most carcinogenic chemicals are genotoxic, but not all genotoxic compounds are carcinogenic for humans. Monitoring of genotoxic effects of chemicals or their mixtures which are present in the environment may be used for the hazard identification and risk assessment. Therefore, the estimations of different endpoints of the genotoxicity are studied to assess the risk of the exposure of humans to particular chemical compounds or their mixtures.

Generally, we have two choices to study the possible genotoxic effect of the environment: we can follow the incidence of genotoxic compounds in the environment by testing the genotoxicity of water, food, air or soil by using different tests for genotoxicity (mostly *in vitro*, as the Ames test, test on cell cultures, etc.); and, the identification of genotoxic agents by chemical analysis may also be useful.

Genotoxicity endpoints are followed directly in exposed humans. The most frequently used endpoints (measured usually in isolated peripheral lymphocytes) are: chromosome aberrations, micronuclei, DNA adducts measured by ^{32}P-postlabelling, DNA damage detected as single or double strand breaks, or alkali-labile sites in the DNA, DNA cross-links, and hypoxantine-guanine phosphoribosyltransferase (HGPRT) mutations.

Methods for the estimation of environmental genotoxicity and methods for the measurements of genotoxicity endpoints in exposed humans are described later in this chapter.

The useful endpoints of genotoxicity are not solely based on biological effects of genotoxins. In some cases, we are not able to measure direct mutagenic effects. However, we can monitor parameters reflecting the exposure to known or unknown mutagens. Thus, we do not measure the genotoxicity itself, but biomarkers of exposure to mutagens. For example, the number of protein adducts may reflect the exposure to a known mutagen that we monitor, but this parameter does not demonstrate anything about the quantitative mutagenic (genotoxic) response in the sense of a biological response.

In some cases it is quite difficult to divide the parameters strictly between the biomarkers of effect and the biomarkers of exposure. DNA adducts are more relevant to biological (genotoxic) effect than are protein adducts; however, they still do not represent the real genotoxic effect because of their possible processing by

DNA repair mechanisms. Similarly, DNA breaks, which we can consider as an effect of mutagens on DNA (from the biochemical point of view), need not necessarily cause mutations.

Other endpoints representing clear parameters of biological effects (chromosome damage or HGPRT mutation induction) can also be regarded as markers of exposure to a mutagen.

The distinction between biomarkers of exposure and genotoxic effects is not definitive. The interpretation of studies using biomarkers depends on the type of biomarker measured. The closer the monitored endpoint is to the biomarker of effect, which is directly related to the disease, the higher the predictive value of the biomarker for the risk assessment. On the other hand, if the biomarker reflects the exposure to a mutagen more than the real effects of the mutagen, it has lower predictive value for the risk assessment, because the exposure to this mutagen may not cause the same effect in all humans due to their different individual susceptibility.

Biomarkers of exposure are usually more specific for certain chemical compounds than are biomarkers of their effects. For example, protein or DNA adducts are measured for specific chemical compounds. On the other hand, biomarkers of effect are not chemical-specific and are less related to the exposure. Gene mutations or chromosome aberrations are not specific for individual chemical compounds.

Various genotoxicity endpoints in tested cells may disappear within different periods of time. This fact may depend on the lifespan of analysed molecules (protein adducts), repair processes (DNA adducts, DNA breaks and cross-links), or on the lifespan of cells (chromosome aberrations). Optimal time after the exposure should be selected for the particular genotoxicity endpoint measurements, depending on the chemical exposure duration and the type of the biomarker followed.

Peripheral lymphocytes isolated from blood samples of humans represent the only biological material which we can obtain from exposed (living) humans in keeping with all ethical rules for work with human tissues. The technique for the blood sampling depends on the particular technique of the assay, requiring special rules for collecting the blood samples. For example, although mostly heparinized blood is used for karyological examination and also for the isolation of lymphocytes for the comet assay, heparin must be avoided in samples used for genotyping. Therefore, separate sample preparations are required for different assays. The type of storage and transport of the sample must be considered from the point of view of the stability of the biomarker (endpoint) measured.

3.3 METHODS FOR THE ASSESSMENT OF GENOTOXIC HAZARDS

3.3.1 Protein Adducts

Mutagenic compounds are known to react with nucleic acid bases in RNA and DNA because of their electrophility. However, not only nucleic acids but also amino acids in proteins contain nucleophilic sites, especially cysteine and histidine. The adducts are estimated mainly in haemoglobin or albumin [1]. N-terminal valine of haemoglobin is very important for the monitoring of protein adduct formation. Because the lifetime of a human erythrocyte is about 4 months, the amount of adducts formed with haemoglobin gives relatively precise information about the exposure of the human to the mutagenic compound [2].

Unlike DNA adducts, there is no repair of protein adducts. Therefore, the kinetics of disappearance are the same as the turnover of the respective protein. For this reason, the optimal collection of blood samples is in

steady state, i.e. balanced between formation and loss of these adducts formed during the chronic exposure. Samples of blood must be transported and stored frozen in tightly closed ampules to prevent any contamination.

The relative advantage of this method is the fact that the extracted dry globulin can be stored for months before the analysis of adducts. S-alkylcysteines and N-alkyhistidines are estimated [3], or the terminal N-alkylvaline is measured by the modified Edman procedure [4, 5].

Albumin adducts are detected in albumin extracted from serum either immunochemically, or using analysis of species released by hydrolysis of the adducted chemical [6]. A statistically different amount of adduct in the exposed population compared to the group of unexposed humans is considered a positive result.

3.3.2 DNA Adducts

A DNA adduct is a chemical entity bound covalently to DNA. Most genotoxic human carcinogens are known to form such adducts with DNA bases. The stability and persistence of adducts in the DNA under physiological conditions is very important and is proportional to their predictive value [7]. The amount of adducts primarily correlates with the exposure, however, the adducts may not correlate with the induction of tumours in a particular tissue [8]. DNA adducts could undergo the process of degradation either spontaneously (as a chemical process), or they can be removed from the DNA by special enzymes involved in the particular pathway of DNA repair. Another reason of a decrease of adducts detected may be the cell loss due to cell turnover, or due to apoptosis or necrosis of heavily damaged cells. The stability of adducts may differ substantially. Adducts formed by aflatoxin B1, for instance, can be highly stable for many days, as well as adducts of O^6-guanine-styrene. Examples of relatively unstable adducts are N^7-guanine or N^3-adenine adduct, whose half-life is only 3 – 5 days [9]. The rapidity by which the particular adducts are removed from the DNA is also different. The cyclobutane-pyrimidine dimers are counted as short-lived, with a half-life of about 15 hours [10], while adducts formed by polycyclic aromatic hydrocarbons have a half-life of >1 month.

Sample collection is optimally made during chronic exposure, similar to sampling for protein adducts. During this exposure, a steady state is achieved between the adduct formation and adduct removal from the DNA. As the removal of adducts can be a relatively rapid process, the sampling should be accomplished within hours after an exposure.

^{32}P post-labelling is the widely used method for the detection of DNA adducts. The advantage of the method is the capability to detect and to quantify modifications of nucleotides. Mass spectrophotometry can be used for the identification of the adduct structure.

Highly purified DNA isolated from the tested tissue or cells (predominantly lymphocytes in the case of human studies) is hydrolyzed to 3´ mononucleotides using micrococcal endonuclease and spleen exonuclease. Because of the vast excess of normal nucleotides in the hydrolysate, the content of adducts is enriched by special methods using P1 nuclease, which cause dephosphorylation of nucleotides at the 3´ end. The dephosphorylation at the 3´ end by P1 nuclease is used to enhance the resolution of adducts.

Most radioactively labelled nucleoside 3´, 5´ diphosphates are separated by polyethyleneimine (PEI) cellulose thin-layer chromatography or high performance liquid chromatography (HPLC). Adducted nucleotides are labelled at the 5´ position with $^{32}P\gamma ATP$ and T4 polynucleotidekinase and the labelled nucleoside 3´, 5´ diphosphate are separated by PEI cellulose chromatography. The resolved adducts are located on PEI cellulose chromatograms using X- ray film and adduct spots are quantified by scintillation counting. Prior to

post-labelling, HPLC is used for the enrichment of small adducts, while bulky adducts are enriched by N-butanol extraction.

As in the case of protein adducts, the optimal time of collection of samples is at the steady state, during long-term exposure. In the case of acute exposure or after termination of chronic exposure, samples should be withdrawn not later than hours or days of the event, because the number of DNA adducts decreases during time due to the repair process [9]. As mentioned above, the DNA level of adducts could be decreased not only due to the DNA repair, but also some adducts (e.g. N^7-guanine, N^3-adenine) are unstable and may be degraded spontaneously, with a halftime of about 3 – 5 days [10].

3.3.3 Chromosome Aberrations

Chromosome aberrations appear as a result of action of a genotoxin reacting with the DNA and causing DNA damage (DNA breaks, or alkali-labile sites – ALS). There are other mechanisms leading to chromosome structural aberrations such as a replication of DNA on the damaged template or inhibition of DNA synthesis. Topoisomerase inhibition may also result in chromosome aberrations. Chromosome aberrations may be followed in peripheral lymphocytes collected from individuals exposed to chemical mutagens.

Ionising radiation and bleomycin induce direct DNA breaks. This kind of DNA damage causes aberration of whole chromosomes (and chromatids) in cells in G0/G1 phase.

In cells treated with a mutagen during the S-G2 phase, aberrations formed involve only one chromatid [11 – 13]. This kind of the DNA damage is detected in lymphocytes after their isolation and stimulation, when they pass through S-phase and mitosis.

When the aberration is caused by DNA synthesis inhibition (without direct DNA damage), it only appears when the inhibitor is present during the S-phase (in vivo). We can barely monitor this type of aberration in stimulated lymphocytes because of the absence of DNA synthesis inhibitor.

Numerical aberrations may occur, leading to aneuploidy or polyploidy. These aberrations are consequences of abnormal cell division and the mechanism is not quite clear. These mechanisms are linked to the damage of mitotic spindle or chromosome structures involved in the mitotic apparatus, etc. [14].

To detect chromosomal aberrations, the dividing cells have to be arrested in mitosis using colchicine which inhibits the inhibition of tubulin polymerization. When lymphocytes are used, these cells have to be stimulated with phytohemagglutinin (PHA).

In the case of acute exposure of humans, it is important to withdraw the lymphocytes as soon as possible after the end of exposure because the persistence of the DNA damage is critical for the frequency of aberrations scored.

The frequency of chromosome aberrations in humans exposed to genotoxic compounds is strongly dependent on the dose, duration and frequency of exposure. The clastogenic mechanism of the tested compound is also important. For example, the frequency of aberrations induced by S-dependent agents can be influenced by the DNA repair, if the DNA damage is repaired before S-phase.

A new technique for the scoring of chromosome aberrations has appeared, enabling the scoring of specific damage in metaphase or interphase cells (including lymphocytes). It is the fluorescence *in situ* method [15 – 19]. This method is better for detection of certain aberrations compared to classic staining methods.

Both structural and numerical aberrations are known to be involved in the etiology of cancer [20, 21]. In general, an increased frequency of chromosome aberrations is associated with an increased risk of cancer [21 – 23].

Blood samples must be heparinised and should be processed within 24 hours; however, lymphocytes can be cultivated successfully even after several days. In the case of late processing, there is a possibility that the DNA damage is repaired prior to the stimulation of lymphocytes to the S-phase. This may explain a decline in the efficiency of recovery of aberrations. Therefore, the blood should be kept at <8^{o}C before processing, and optimally at 2^{o}C.

Whole blood is added to the cultivation medium containing PHA (phytohemagglutinin) and cultures are incubated for 1 generation (until the first mitosis), when the highest frequency of aberrations appears. During mitotic division, the appearance of aberrations is significantly decreased because the damage is diluted among daughter cells. The more damaged cells may be lost, and a decrease of the aberration frequency may also occur. Therefore, the cultures are harvested 48 hours after stimulation with PHA to obtain the first generation of lymphocytes.

As the proliferation of the first and second generations of lymphocytes may vary among individuals, the chromatids are labelled by adding BUdR into the culture medium to discern the first and the second mitosis [24]. Before harvesting, colcemide is added for the last 2 hours of cultivation. The routine processing of cells for the preparation of karyological preparations follows, using the hypotonization of cells (0.075 M KCL) and fixation 3:1 (methanol : acetic acid). Slides are prepared and cells stained with differential staining for metaphase cells based on the incorporation of BUdR.

Aberrations are scored using human cytogenetic nomenclature [25]. Chromosomal damage such as breaks are regarded as a consequence of breakage of DNA, while large rearrangements of chromosomes involve multiple breakages and misrepair. In addition to the classical scoring, the FISH method can be used for the evaluation of structural aberrations. Differential staining of 2 – 6 chromosome pairs is carried out by means of special DNA probes.

The frequency of aberrations measured must be compared with a control or reference population to determine meaningful differences for individuals.

3.3.4 Micronuclei

Micronuclei (MN) arise in mitotic cells from fragmented chromosomes. Some chromosomes may lag behind during anaphase and are not integrated in the nuclei of daughter cells. Such MN may appear as a consequence of DNA breakage, replication on the damaged DNA or inhibition of DNA synthesis. MN containing whole chromosomes also exist. They can be formed by the failure of mitotic spindle, kinetochore, or chromosome structures taking part in the chromosome movement. The frequency of MN can represent not only the active DNA breakage but also reflects an aneuploidogenic effect which is not linked to effects on DNA.

The frequency of MN in human studies is determined in stimulated lymphocytes, similarly to chromosome aberrations [26]. MN arising from lagging chromosomes may contain kinetochores which may be clearly identified by a specific antibody. They also contain centromeres which can be identified by selective DNA probes against centromere DNA sequences.

Like chromosome aberrations, the frequency of MN appearing in the studied population must be compared with control or reference populations. There should be a clear dose-response which helps support the significance of results.

Lymphocyte cultures are prepared by similar techniques to those used for chromosome aberrations scoring (see above) and stimulated by phytohemagglutinin. The analysis is limited to cells that have divided once *in vitro*. The technique uses cytochalasin B (cyt B) added to the culture to block cytokinesis in the telophase [27]. Therefore, cells containing 2 nuclei can be identified, representing cells which have divided once *in vitro*. The proliferation activity of cells can also be scored by this mechanism. Binucleated cells are only scored for MN with this cytochalasin B block-method.

3.3.5 Single Cell Gel Electrophoresis (SCGE) – Comet Assay

We can estimate the DNA damage in terms of single strand breaks (SSB) in any accessible cells which can be isolated or prepared as a single cell suspension by this technique [28 – 31]. In this assay, cells are embedded in agarose on microscopic slides and lysed in lysing solution containing detergent and high salt. There are neutral and alkaline versions of the method. In the alkaline version of this method, lysed cells are placed in alkaline solution (0.3 M NaOH) to unwind DNA. DNA is pulled to the anode during electrophoresis. If the DNA contains alkali-labile sites (ALS), it is released from the nucleoids and moves to the anode, forming a comet-like pattern in the fluorescent microscope after staining of the DNA with fluorescent dyes. In the case of neutral version of the method, the alkaline unwinding is avoided and the electrophoresis is carried out under the neutral pH. Under neutral pH, only double strand breaks can cause the formation of the comet. On the other hand, the single strand and double strand breaks may be analyzed at pH 12.1. At higher pH, both single strand breaks and alkali-labile sites (ALS) in the DNA molecule (which disrupt and form breaks under the high pH) are detected. Strand breaks may arise from direct strand breakage or as an intermediate stage during incomplete DNA repair.

Modifications of the technique have been published enabling the detection of specific DNA lesions (pyrimidine dimmers, oxidised bases, alkylated bases) using the cleaving of DNA in nucleoids by specific enzymes (like T4 endonuclease, endonuclease III, formamido- pyrimidine nuclease) [32, 33].

After the electropohoresis, cells are stained with fluorescent dyes staining the DNA such as DAPI, ethidium bromide or acridine orange and several parameters are evaluated using the image analysis software. The comet tail length (TL), % of DNA in tail (TD) and tail moment (TM) are the most important parameters [34, 35].

3.4 TESTING OF ENVIRONMENTAL CONTAMINATION WITH GENOTOXIC AGENTS

Generally, air, water and soil can be contaminated with different genotoxic agents. Some of these agents may be present naturally such as different heavy metals, etc. Other genotoxic contamination of environment may be a consequence of human activity, especially as products of industry. Many chemicals arising from industrial activity may contaminate the environment as air pollution or wastewater.

Any human activity in heavily polluted areas may represent a health risk. Military activities in such areas may also increase health risks to soldiers, especially during long-term missions. Therefore, the monitoring of genotoxicity of water, air and eventually soil may be very useful for the prevention of any influence of such pollutants on health of military personnel operating in polluted areas and will enable health risk assessment.

As a consequence of industrial activity, many chemicals may spread into the surroundings. When these chemicals are stable, such as DDT, PCBs or heavy metal salts, they may stay in the environment for years and so

they can influence organisms living in contaminated areas, including man. These chemicals may contaminate water or may get into the food chain; thus representing a risk in the case of the long-term exposure of humans.

Military members may be at special risk for certain chemical exposures, such as those coming from the use of chemical weapons or explosives, diesel or other military engines, and fires in or near the battlefield (e.g. as a consequence of enemy activity – see burning oil fields in Iraq during the Gulf war). Many other potentially hazardous chemicals may be encountered during missions, e.g. insecticides, defoliants, disinfection, detergents, etc.

All these agents, or environmental contamination by them, should be under control to minimise their harmful influence on the health of soldiers or inhabitants in the area, and to enable risk assessment in case of human exposure to them. Thus, testing the environment for genotoxicity should be conducted in the operational areas of deployed military troops, especially in areas in which a long-term stay is expected (e.g. military bases, camps, etc.). The testing should especially examine water, air and soil. Techniques and methods for such testing have been used in many cases of industrial pollution and chemical contamination of the environment.

3.4.1 Air Genotoxicity

Any combustion process may increase the genotoxicity of the air. A typical source is the coal-burning power plant, but local heating of houses by burning of contaminated fuels may also substantially pollute the air. Any type of dust is also important. The dust particles may absorb different compounds such as aromatic hydrocarbons. Results of many studies focused on the genotoxicity of the air have been published. These studies are focused on several types of air pollution. In these studies, the biological activities of complex mixtures of organic compounds adsorbed onto ambient airborne dust particles are evaluated. Particles are collected on special teflon-coated filters and later extracted with dichloromethane or other solvents. The genotoxicity of compounds contained in dichloromethane extractable fraction is then measured. Primarily, the following short-term in vitro assays are used [36, 37]:

- Ames test. Bacterial mutagenicity test using different Salmonella typhimurium strains TA98 and YG1041.
- DNA adducts estimation using 32P-postlabelling. The induction of DNA adducts is measured in target human or mammalian cells treated in vitro with extracts from air samples (primarily HePG2 cells or isolated rat hepatocytes are used, because they are able to activate phenylalanine hydroxylase (PAH)).
- DNA breaking activity of air extracts. In this case, target cells are treated with air sample extracts dissolved in DMSO for different period of time (usually 1 – 3 hours) and the resulting DNA breaks in cells are scored using the alkaline version of the comet assay.
- Plant-based tests are also used for the evaluation of air-genotoxicity. As an example, Tradescancia growing in the tested area and the induction of micronuclei (MN) is one type of assay [38].

3.4.2 Water Genotoxicity

The water may be also tested by similar methods [39]. First of all, the induction of bacterial mutation in the Ames test is suitable, supplemented with S9 fraction isolated from rat liver. The induction of the DNA damage in cultured mammalian cells detectable with comet assay is important information about the possible action of the genotoxic chemical compound. Similar to air testing, some assays based on the use of plants may also be used.

3.4.3 Soil Genotoxicity

The genotoxicity of soil is important from the point of view of long-term missions, when the soldiers may be accommodated in the confined geographical area of the base for a long time. Thus, they may be exposed not only to the effects arising from industrial pollution, but also to the contamination of water with different PAH and to hydrocarbons from fuel which may be quite highly concentrated in contaminated soil.

Tests are aimed at evaluating the genotoxicity of contaminated soils by means of an integrated chemical/ biological approach, using a short-term bacterial mutagenicity test (Ames test), a plant genotoxicity test (*Tradescantia*/micronucleus test), and chemical analyses. The genotoxicity of soil is measured mostly using water or dichlormethane extracts from the soil [40]. Similar to the testing of air and water, the induction of DNA damage (strand breaks) in cultured rat or human cells exposed to soil extract may be followed using the comet assay.

3.5 REFERENCES

[1] National Research Council USA, *Biological Markers In Reproductive Toxicology*, Washington, D.C.; National Academy Press, 1989.

[2] International Programme on Chemical Safety IPCS. *Biomarkers And Risk Assessment: Concepts And Principles*. Environmental Health Criteria, 155, Geneva, Switzerland; World Health Organization, 1993.

[3] Council for International Organizations of Medical Sciences. *International Ethical Guidelines for Biomedical Research Involving Human Subjects*. Geneva, Switzerland; CIOMS/WHO, 1993.

[4] World Medical Association. Declaration of Helsinki. *JAMA*, 1997; 277:925-926.

[5] Sample Power 1.2 software, Chicago, IL; SPSS, Inc.

[6] Tates, A.D., Grummt, T., Van Dam, F.J., deZwart, F., Kasper, F.J. and Rothe, R., et al. Measurement of frequencies of HPRT mutants, chromosomal aberrations, micronuclei, sister-chromatid exchanges and cells with high frequencies of SCEs in styrene r dichloromethane-exposed workers. *Mutat. Res.* 1994; 313:249-262.

[7] Qian, G.S., Ross, R.K., Yu, M.C., Yuan, J.-M., Gao, Y.-T. and Henderson, B.E., et al. A follow-up study of urinary markers of aflatoxin exposure and liver cancer risk in Shanghai, People's Republic of China. *Cancer Epidemiology Biomarkers and Prevention* 1994; 3:3-10.

[8] Culp, S.J., Gaylor, D.W., Sheldon, W.G., Goldstein, L.S. and Beland, F.A. DNA adduct measurement in relationship to small intestinal and forestomach tumour incidence during the chronic feeding of coal tar or benzpyrene to mice. *Polycyclic Aromatic Hydrocarbons* 1996; 11:161-168.

[9] McGregor, D. and Anderson, D. DNA damage and repair in mammalian cells in vitro and in vivo as indicators of exposure to carcinogens. in: McGregor DB, Rice JM, Venitt S Eds. *IARC Scientific Publications 146. The use of short-term medium tests for carcinogens and data on genetic effects in carcinogenic hazard evaluation*. Lyons, France; International Agency for Research on Cancer, 1999; 309-354.

[10] Zhao, C., Kumar, R., Zahlsen, K., Bager-Sundmark, H., Hemminki, H. and Eide, I. Persistence of 7-2-hydroxyethyl guanine-DNA adducts in rats exposed to ethene by inhalation, *Biomarkers* 1997; 2:355-359.

[11] Kihlman, B.A. *Caffeine and Chromosomes*. Amsterdam, the Netherlands; Elsevier, 1977.

[12] Buckton, K.E. and Evans, H.J. Eds. *Methods for the analysis of human chromosome aberrations.* Geneva, Switzerland; World Health Organization, 1973.

[13] Evans, H.J. and O'Riordan, M.L. Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen test. *Mutat Res* 1975; 31:135-148.

[14] Oshimura, M. and Barrett, J.C. Chemically induced aneuploidy in mammalian cells: mechanisms and biological significance in cancer. *Environ Mutagen* 1986; 8:129-159.

[15] Pinkel, D., Straume, T. and Gray, J.W. Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridisation. *Proc. Natl. Acad. Sci. U. S. A*., 1986; 83:2934-2938.

[16] Eastmond, D.A. and Pinkel, D. Detection of aneuploidy and aneuploidy-inducing agents in human lymphocytes using fluorescence in situ hybridisation with chromosome-specific DNA probes. *Mutat Res* 1990; 234:303-318.

[17] Eastmond, D.A., Schuler, M. and Rupa, D.S. Advantages and limitations of using fluorescence in situ hybridisation for the detection of aneuploidy in interphase human cells. *Mutat Res* 1995; 348:153-162.

[18] Rupa, D.S., Hasegawa, L. and Eastmond, D.A. Detection of chromosomal breakage in the 1cen – 1q12 region of interphase human lymphocytes using multicolor fluorescence in situ hybridisation with tandem DNA probes. *Cancer Res* 1995; 55:640-645.

[19] Boei, J.J.W.A., Vermeulen, S. and Natarajan, A.T. Detection of chromosomal aberrations by fluorescence in situ hybridisation in the first three postirradiation divisions of human lymphocytes. *Mutat Res* 1996; 349:127-135.

[20] Mitelman, F. Chromosomes, genes, and cancer. Editorial. *CA Cancer J Clin* 1994; 44:133-135.

[21] Hagmar, L., Brogger, A., Hansteen, I.L., Heim, S., Hogstedt, B. and Knudsen, L., et al. Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. *Cancer Res* 1994; 54:2919-2922.

[22] Bonassi, S., Abbondandolo, A., Camurri, L., Dal Pra, L., De Ferrari, M. and Degrassi, F., et al. Are chromosome aberrations in circulating lymphocytes predictive of a future cancer onset in humans? Preliminary results of an Italian cohort study. *Cancer Genet Cytogenet* 1995; 79:133- 135.

[23] Hagmar, L., Bonassi, S., Stromberg, U., Brogger, A., Knudsen, E., Norppa, H. and Reuterwall, C. Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health ESCH, *Cancer Res* 1998; 58:4117-4121.

[24] De Boer, P., Van Buul, P.P.W., Van Beek, R., Van der Hoeven, F.A. and Natarajan, A.T. Chromosomal radiosensitivity and karyotype in mice using cultured peripheral blood lymphocytes and comparison with this system in man. *Mutat Res* 1977; 42:379-394.

[25] Harnden, D.G. Ed. *ISCN, An International System for Human Cytogenetic Nomenclature*. Basel, Switzerland; S. Karger, 1985.

[26] Fenech, M. and Morley, A. Measurement of micronuclei in lymphocytes. *Mutat Res* 1985; 147:29-36.

[27] Abdel-Rahman, S.Z., el-Zein, R.A., Zwischenberger, J.B. and Au, W.W. Association of the NAT1*10 genotype with increased chromosome aberrations and higher lung cancer risk in cigarette smokers. *Mutat Res* 1998; 398:43-54.

[28] Tice, R.R. The single cell gel comet assay: a microgel electrophoretic technique for the detection of DNA damage and repair in individual cells. In: Phillips DH, Venitt S, Eds. *Environmental Mutagenesis*. Oxford, UK; Bios Scientific Publishers, 1995; 315-339.

[29] Anderson, D., Yu, T.-W. and McGregor, D.B. Comet assay responses as indicators of carcinogen exposure. *Mutagenesis* 1998; 13:539-555.

[30] Pool-Zobel, B.L., Lotzmann, N., Knoll, M., Kuchenmeister, F., Lambertz, R. and Leucht, U., et al. Detection of genotoxic effects in human bystric and nasal mucosa cells isolated from biopsy samples. *Environ Mol Mutagen*1994; 24:23-45.

[31] Anderson, D., Dobrzynska, M.M. and Basaran, N. Effect of various genotoxins and reproductive toxins in human lymphocytes and sperm in the comet assay. *Teratogen Carcinog Mutagen* 1997; 17:29-43.

[32] Collins, A.R., Duthie, S.J. and Dobson, V.L. Direct enzymatic detection of endogenous base damane in human lymphocyte DNA. *Carcinogenesis* 1993; 14:1733-1735.

[33] Collins, A.R., Dusinska, M., Gedik, C.M. and Stetina, R. Oxidative damage to DNA: do we have a reliable biomarker? *Environ Health Perspect* 1996; 104:465-469.

[34] Olive, P.L. and Banath, J.P. Induction and rejoining of radiation-induced DNA single strand breaks: ‘‘tail moment’’ as a function of position in the cell cycle. *Mutat Res* 1993; 294:275-283.

[35] Hellman, B., Vaghef, H. and Bostrom, B. The concepts of tail moment and tail inertia in the single cell gel electrophoresis assay. *Mutat Res* 1995; 336:123-131.

[36] Binkova, B., Cerna, M., Pastorkova, A., Jelínek, R., Benes, I., Novak, J. and Sram, R.J. Biological activities of organic compounds adsorbed onto ambient air particles: comparison between the cities of Teplice and Prague during the summer and winter seasons 2000-2001. *Mutat Res* 2003; 9:43-59.

[37] Karlsson, H.L., Nygren, J. and Miller, L. Genotoxicity of airborne particulate matter: the role of cell-particle interaction and of substances with adduct-forming and oxidizing capacity. *Mutat Res* 2004; 31:1-10.

[38] Klumpp, A., Ansel, W., Klumpp, G., Calatayud, V., Garrec, J.P., He, S., Peñuelas, J., Ribas, À., Ro-Poulsen, H., Rasmussen, S., Sanz, M.J. and Vergne, P. Tradescantia micronucleus test indicates genotoxic potential of traffic emissions in European cities. *Environ Pollut* 2006; 139:515-522.

[39] Cerna, M., Pastorkova, A., Bavorova, H., Ocadlikova, D., Smid, J. and Rössner, P. Genotoxicity of complex and fractionated wastewater discharges and river waters. *Mutation Research/Environmental Mutagenesis and Related Subjects*. 1996; 360:274-275.

[40] Monarca, S., Feretti, D., Zerbini, I., Alberti, A., Zani, C., Resola, S., Gelatti, U. and Nardi, G. Soil contamination detected using bacterial and plant mutagenicity tests and chemical analyses. *Environ Res* 2002; 88:64-69.

Chapter 4 – MONITORING OF AIR IN CONFINED AND OPEN SPACES

by

M.A.E. Mol, M.M. van Deursen, F.R. Groeneveld and H.C. Trap
TNO Defence, Security and Safety, Rijswijk, Netherlands

Recent military operations have highlighted the problem of possible chemical hazard exposure in troops. A risk assessment process is especially important for military forces deployed in chemically contaminated environments, and measurement of exposures will help in preventing or reducing incapacitation during deployment or development of disease after deployment [1 – 4]. Therefore, a need exists in operational military settings to rapidly detect a wide range of chemicals with potential adverse health effects for exposed personnel. Exposure to airborne contaminants that arise from occupational military activities and local environmental pollution is a major contributor to health problems. Inhalation of gases, vapors, aerosols, and mixtures of these can cause a wide range of adverse health effects, ranging from simple irritation to debilitating systemic diseases. In operational military settings, monitoring of levels of hazardous air pollutants at deployment sites occurs before and during deployment. Insight into the quality of the air at the deployment location is of particular importance, since whereas one may choose to import drinking water and food from the homeland one has to breathe the air that is locally present. It is unlikely that military missions of several months will be fulfilled while continuously wearing respiratory protection.

Pre-deployment screening of air will give a first impression of the quality of the ambient atmosphere for a variety of toxic industrial compounds at a future camp site. Data collected during this quick scan contribute to the choice for the best location for future settlement. An inventory of equipment fit for performing of quick scans was made recently by van Deursen et al. [5]. Performance of such scans is limited by transportation opportunities to the survey site, time available to be spent there, and the type of equipment which can be used. The gas detection instruments should be portable (10 – 15 kg) at least, but ideally hand-held (max. 2 kg). Various hand-held instrumentation to detect toxic air hazards are commercially available, based on different technologies such as electrochemical cells, detector tubes, photo ionization detectors or ion mobility spectrometry. Hand-held devices are easy to use and have a fast response; they detect most of the immediately irritating gases. Often only single gases can be detected at a time, but with some multiple-gas analyzers four or five components can be detected simultaneously. The detection limits of hand-held devices are in the low ppm-range. This type of device is suitable if one wants to screen roughly for known toxic compounds.

More detailed information on airborne contaminants can be obtained by using portable devices based on infra-red technology (e.g. Miran SapphIRe and HazMatID Portable Chemical Identifier), and on gas chromatography-mass spectrometry (GC-MS). Instruments based on the latter technology have the largest potential for identification of a broad range of chemicals at ppb or even lower levels. The HapSite is a good example of portable GC-MS equipment that can be used in field situations such as the pre-deployment quick scan.

During the quick scan, additional environmental air samples can be collected for off-site analysis in a (mobile) laboratory at a distance, yielding more sensitive analyses with sophisticated laboratory facilities. Sampling techniques and transport conditions are key factors that determine the outcome of off-site analyses. To cover most air contaminants three ways of sampling are recommended [6]. Adsorption on solid adsorbent cartridges, e.g. Tenax, is the best way to catch volatile organic compounds (VOC), whereas gases can be sampled best in Tedlar gas bags. For analysis of particulate matter, air should pass through filters to collect the particles.

Compounds collected in this way remain stabile when stored at refrigerated temperatures for not longer than 7 days.

At a laboratory, air samples can be analyzed by one-dimensional gas chromatography (GC) coupled to various detectors such as a flame ionization detector (FID), a flame photometric detector (FDP) or a nitrogen phosphor detector (NPD). Direct identification of the compounds may occur by GC-MS. Furthermore, a Fourier Transform Infra Red (FT-IR) gas-analyzer and NOx analyzer with chemiluminescence monitor may be supplementary to perform standard analyses for gases and vapors [7]. However, airborne mixtures often are complex, such as atmospheres produced by combustion – e.g. diesel exhaust and residual oil fly ash – and the analysis of VOC is a challenge. VOC are associated with adverse effects to human health; at low concentrations they can cause cancer, immunological and neurological damage, as well as reproductive and endocrine disorders. However, it is often difficult to determine these toxic compounds at trace concentrations due to lack of resolution and sensitivity in single-column GC analysis. The toxicants have to be separated from an excess of other compounds present at many orders of magnitude of higher concentrations. Therefore, the analytical procedures employed have to be highly selective and sensitive. Comprehensive two-dimensional gas chromatography (GC×GC) may offer a solution to that problem, because it offers enhanced resolution for complex mixtures containing trace level environmental toxicants. Several studies demonstrated that GC×GC is a promising technique for analysis of complex environmental toxicants [8].

During deployment, air quality sampling can be done on-site at the camp in a mobile lab with portable devices or with appropriate equipment as previously described. The acute toxicity of many organic vapors means that close to real-time air monitoring is desired to minimise risk to human health. In addition, samplers to measure the presence of coarse particulate matter (PM10) at the campsites and outside the camps during patrols are required. Dust exposure is a primary disease and non-battle injury threat in countries where natural ambient air levels for PM10 (265 to over 670 $\mu g/m^3$) exceed by far the safety standard of western countries (70 $\mu g/m^3$) [9 – 10].

Although suitable analytical methods are available to identify numerous individual contaminants in air, meaningful risk assessment of a complex mixture such as polluted inhaled air is a major challenge [11 – 12]. Individual risk assessment of the constituents of air pollution disregards possible combinatory effects. Risk assessment of a complex mixture of air pollutants on the basis of individual compounds is not yet possible since no adequate procedures are available to integrate toxicity data of complex mixtures.

As an alternative for the measurement of the toxicological effects of mixtures of gases, vapors, and particles that actually occur in environmental air, a biologically-based toxicity monitoring system might be used. Using a biological sentinel, toxicity caused by unsuspected materials as well as by the interactions of chemicals in complex mixtures can be detected. Several field portable monitoring systems are available, but they are exclusively designed to observe the quality of aqueous samples. They are whole-cell- or organism-based and monitor the biological effects of contaminants. Recently, the US Army Center for Environmental Health Research has developed an aquatic biomonitoring system based on bluegills for real time toxicity detection of toxic conditions caused by a wide range of chemicals or chemical mixtures. They are also involved in studies showing that the ECIS biosensor (Agave BioSystems) can be used to observe toxic conditions in aqueous samples. The ECIS biosensor is currently being adapted for use as a field portable system for real-time air toxicity monitoring [13] .In cooperation with the University of Montana, USACEHR is exploring the potential of using bees for real-time air monitoring. In this study, volatile and semivolatile chemicals in the air inside beehives are detected and the responses of honeybee colonies to harmful substances are observed in electronic beehives [14].

On a laboratory scale, other systems are under development to detect the toxic effects of chemical mixtures in air on a biological system. Two commercial exposure chamber devices, the Harvard/Navicyte horizontal diffusion chambers and the CULTEX system are used for monitoring the response of cultured cells to complex air exposures like polluted ambient air, cigarette smoke and diesel exhausts [15 – 18]. To achieve a continuous direct exposure to gases, cells are cultured at the air-liquid interface on microporous membranes. These bio-based systems can rapidly provide information on air toxicity caused by gases and vapors as well as by aerosols.

4.1 REFERENCES

[1] Rossi, J. 3rd, Ritchie, G.D., Nordholm, A.F., Knechtges, P.L., Wilson, C.L., Lin, J., Alexander, W.K. and Still, K.R. Application of neurobehavioral toxicology methods to the military deployment toxicology assessment program. *Drug Chem Toxicol* 2000; 23:113-38.

[2] Still, K.R., Jederberg, W.W., Ritchie, G.D. and Rossi, J. 3rd. Exposure assessment and the health of deployed forces. *Drug Chem Toxicol* 2002; 25:383-401.

[3] Hauschild, V.D. and Lee, A.P. Assessing chemical exposures during military deployments. *Mil Med* 2004; 169:142-6.

[4] Hauschild, V.D. and Bratt, G.M. Prioritizing industrial chemical hazards. *J Toxicol Environ Health* A 2005; 68:857-76.

[5] Van Deursen, M.M., Groeneveld, F.R. and Zappey, H.W. Inventarisatie van technologieën en apparatuur voor het uitvoeren van een quick-scan. *TNO-Report*, in preparation 2006.

[6] Van Deursen, M.M., Groenveld, F.R., van Zuijlen, G.A. and Trap, H.C. Monstername en analyse van toxische stoffen (een evaluatie van off-site analyses van omgevingslucht). *TNO Report TNO-DV2 2005 A079*, Rijswijk, Netherlands; TN, 2005.

[7] Groeneveld, F.R. Optimalisatie van analysemethoden voor toxische componenten in gassen. *TNO-Report*. In preparation 2006.

[8] Reichenbach, S.E., Ni, M., Kottapalli, V., Visvanathan, A., Ledford, E.B., Oostdijk, J. and Trap, H.C. Chemical Warfare Agent (CWA) Detection with Comprehensive Two-Dimensional Gas Chromatography (GCxGC). *Proceedings SPIE Conference on Chemical and Biological Sensing* IV 2003; 5085:28-36.

[9] Van Hook, D., Colonel US Air Force, personal communication. Third Meeting of NATO HFM-057/ RTG-009 on Protection against Adverse Effects of Toxic Hazards. Regional Medical Command Headquarters, Schloss Oranienburg, Diez, Germany, 25-26 June 2002.

[10] Hommes, C., Royal Dutch Navy, personal communication. Third Meeting of NATO HFM-057/RTG-009 on Protection against Adverse Effects of Toxic Hazards. Regional Medical Command Headquarters, Schloss Oranienburg, Diez, Germany, 25-26 June 2002.

[11] de Rosa, C.T., El-Masri, H.A., Pohl, H., Cibulas, W. and Mumtaz, M.M. Implications of chemical mixtures in public health practice. *J Toxicol Environ Health B Crit Rev*. 2004; 7(5): 339-50.

[12] Bjarnason, S.G. Toxicology of chemical mixtures: a review of mixtures assessment. In: *Biotechnologies for Assessment of Toxic Hazards in Operations Environments* (DRDC TM 2004-016. Suffield, Canada; Defence Research and Development Canada, November 2004.

[13] www.agavebio.Com – (Last accessed 31 January 2007).

[14] www.usacehr.org – (Last accessed 31 January 2007).

[15] Aufderheide, M. Direct exposure methods for testing native atmospheres. *Exp Toxicol Pathol* 2005; 57 Suppl 1:213-26.

[16] Bakand, S., Winder, C., Khalil, C. and Hayes, A. Toxicity assessment of industrial chemicals and airborne contaminants: transition from in vivo to in vitro test methods: a review. *Inhal Toxicol* 2005; 17:775-87.

[17] Fukano, Y., Ogura, M., Eguchi, K., Shibagaki, M. and Suzuki, M. Modified procedure of a direct in vitro exposure system for mammalian cells to whole cigarette smoke. *Exp Toxicol Pathol* 2004; 55:317-23.

[18] Fukano, Y., Yoshimura, H. and Yoshida, T. Heme oxygenase-1 gene expression in human alveolar epithelial cells (A549) following exposure to whole cigarette smoke on a direct in vitro exposure system. *Exp Toxicol Pathol* 2006; 57:411-8.

Chapter 5 – TOXICOLOGY OF MIXTURES: A REVIEW OF MIXTURES ASSESSMENT

by

S.G. Bjarnason

Defence R&D Canada, Suffield, Canada

A presentation based on the report was made to NATO HFM-057/RTG-009 (Protection Against Adverse Effects of Toxic Hazards) in Delft, The Netherlands, 19 February 2004. This report has also appeared as a Technical Memorandum issued by Defence R&D Canada, Suffield.

5.1 ABSTRACT/RESUME

The science of risk assessment revolves broadly around hazard identification (toxicity) and exposure assessment information. While exposure to environmental hazards most often occurs with complex chemical mixtures, the majority of existing toxicity data is for single compounds or simple mixtures, thus presenting problems to the risk assessor. Several approaches to assess mixtures have been developed (e.g. Hazard Index; Target-organ Toxicity Dose; Weight-of Evidence; Toxic Equivalence), each of which have their limitations, primarily with respect to the prediction of potentially unforeseen interactions between the mixture constituents which may affect their resultant toxic outcome. Recent advances in disciplines such as genomics, proteomics, metabonomics and physiologically-based pharmacokinetic modeling should assist in the hazard assessment of complex chemical mixtures. However, the process of regulatory assessment of these types of exposures will remain both complex and difficult.

La science de l'évaluation du risque comprend globalement l'identification du danger (toxicité) et des informations sur les possibilités d'exposition. Alors que des mélanges chimiques complexes sont impliqués le plus souvent dans les risques environnementaux, la majorité des données de toxicité existante concerne des composés isolés ou des mélanges simples, constituant ainsi un problème pour le responsable de l'évaluation des risques. Plusieurs approches ont été développées pour évaluer les mélanges (par exemple l'index de risques, la dose toxique pour organe cible, le poids de la preuve ou l'équivalence toxique). Chacune possède ses limites, principalement lors de la prédiction de possibles interactions non envisagées entre les constituants du mélange, interactions qui peuvent modifier le pouvoir toxique final. Les avancées récentes dans des disciplines telles que la génomique, la protéomique, la métabonomique et la pharmacocinétique utilisant des modèles physiologiques devraient rendre plus aisée l'évaluation du risque constitué par les mélanges chimiques complexes. Cependant, le processus d'évaluation réglementaire de ce type d'exposition restera à la fois complexe et difficile.

5.2 EXECUTIVE SUMMARY

Exposure to environmental contaminants occurs to mixtures and not to single contaminants. These exposures may also occur across multimedia environments, soil, water and air, which significantly complicates an assessment of their impact on health. The problems of assessing environmental contaminants are two-fold; understanding the potential effects and determining exposure concentration. The toxicology of complex mixtures is very poorly understood and the capture of relevant exposure information for mixtures has been a problem. This paper focuses on chemical hazards and how different agencies assess mixtures in occupational

and environmental settings. Both exposure scenarios were examined. Troops on deployment may be faced with occupational levels of exposure to mixtures, but the length of exposure may be closer to that evaluated for an environmental setting (i.e. 24 hours per day, 7 days per week).

Very few mixtures have been studied as complete mixtures in a laboratory setting; therefore several evaluation approaches have been developed. These methods are based on toxicological information that is known for the constituent compounds, as well as available exposure information. Each approach makes certain assumptions to reach a conclusion and these are often a limiting factor in the utility of the approach. These approaches are used by governmental and non-governmental agencies around the world for occupational and environmental assessments of mixtures.

Occupational exposure assessment generally assumes a higher dose or exposure level than an ambient or environmental assessment because the former is generally for a specified time period (e.g. 8 hours/day; 5 days/week) in a very specific environment. Occupational health organizations have developed evaluation systems for both non-cancer causing and cancer causing mixtures using different assessment schemes based on the level of available information. Agencies evaluating environmental exposures have developed similar assessment tools, but the exposure levels are lower and the time period is continuous (i.e. 7 days per week). As with occupational assessments, different schemes are used for cancer and non-cancer causing components of complex mixtures.

Assessment of complex chemical mixtures is hampered by the lack of toxicity data on complete mixtures, as well as the potential for chemical interactions within the mixture that may result in unforeseen by-products that have an inherent toxicity. Another factor that is not generally accounted for is the effect of stress on exposure and resultant toxicity, and also of exposure-induced stress.

There is significant effort being applied to develop an understanding of how complex mixtures should be assessed and these efforts are being led by civilian agencies and organizations. The information, guidelines and regulations coming from these efforts can act as a guide to assist in developing methodologies to assess risk to troops in different chemical environments.

L'exposition aux contaminants environnementaux existe au contact de mixtures et non d'un seul contaminant. Ces expositions peuvent aussi exister dans des milieux multiples, des sols, l'eau et l'air ce qui complique l'évaluation de leur impact sur la santé. Le problème de l'évaluation des contaminants environnementaux présente deux aspects : la compréhension des effets potentiels et la détermination de la concentration de l'exposition. La toxicologie des mixtures complexes est très peu comprise et la sélection de l'information pertinente concernant les mixtures a toujours posé un problème. Cet article se concentre sur les dangers chimiques et sur la façon dont les agences évaluent les mixtures dans des contextes professionnels et environnementaux. Les deux scénarios d'exposition ont été examinés. Les troupes déployées peuvent avoir à faire face à l'exposition aux mixtures dans le contexte de leur profession mais la durée de l'exposition peut être plus proche de celle évaluée pour des contextes environnementaux (par ex. : 24 h par jour, 7 jours par semaine).

Très peu de mixtures ont été étudiées dans leur totalité en laboratoire, par conséquent plusieurs méthodes d'évaluation ont été développées. Ces méthodes sont basées sur l'information toxicologique connue pour les composés ainsi que sur l'information disponible concernant l'exposition. Chaque méthode atteint une conclusion à partir de certaines assomptions ce qui est souvent un facteur limitatif quant à l'utilité de la méthode. Ces méthodes sont utilisées par les agences gouvernementales et non gouvernementales du monde entier en ce qui concerne l'évaluation de ces mixtures dans le contexte professionnel et environnemental.

L'évaluation de l'exposition dans le contexte professionnel considère, en règle générale, que la dose ou le niveau d'exposition est plus important que pour une évaluation environnementale parce que la première évalue normalement une durée de temps spécifique (par ex. : 8 heures par jour ; 5 jours par semaine) dans un milieu très spécifique. Les organisations de la santé du travail ont développé des systèmes d'évaluation pour des mixtures qui causent le cancer et pour celles qui ne le causent pas, utilisant des stratagèmes d'évaluation différents basés sur le niveau d'information disponible. Les agences évaluant les expositions environnementales ont développé des outils d'évaluation similaires mais les niveaux d'exposition sont plus bas et la durée de temps est continue (par ex. : 7 jours par semaine). Tout comme pour les évaluations dans le contexte d'une profession, des stratagèmes différents ont été utilisés pour les composants de mixtures complexes causant et ne causant pas le cancer.

L'évaluation de mixtures chimiques complexes est retardée par le manque de données concernant la toxicité de la totalité des mixtures ainsi que par le potentiel d'interaction chimique entre la mixture pouvant résulter de sous-produits imprévus qui possèdent une toxicité inhérente. Un autre facteur dont on ne tient normalement pas compte est l'effet du stress sur l'exposition et la toxicité qui en résulte ainsi que du stress induit par l'exposition.

On s'efforce principalement de comprendre comment les mixtures complexes devraient être évaluées et ces efforts sont conduits par des agences et des organisations civiles. L'information, les lignes directrices et les règlements qui résultent de ces efforts servent de guide au développement des méthodologies et à l'évaluation du risque posé aux troupes se trouvant dans des milieux chimiques différents.

5.3 INTRODUCTION

The science of risk assessment revolves broadly around hazard identification (toxicity) and exposure assessment information. While exposure to environmental hazards most often occurs with complex chemical mixtures, the majority of existing toxicity data is for single compounds or simple mixtures, thus presenting problems to the risk assessor. For decades now, regulators worldwide have grappled with the complexities of risk assessing chemical mixtures [1 – 3].

Xenobiotic exposures commonly occur across multimedium environments, including water, air and soil, which act to modulate the actual dose received by the exposed individual. Simple everyday activities such as bathing, eating, drinking and moving from an indoor to outdoor environment complicate the exposure assessment to even single toxic compounds. The adequate capture of such exposure data has been an ongoing problem and the development of sensitive and accurate personal dosimetry is an active field of study. Further complicating exposure assessment is the necessity of understanding the environmental fate of the compounds in question; how they interact in and with the environment can alter both the toxicity and exposure scenarios. An example of this is the combination of volatile organic compounds with oxygen and sunlight to produce ground level ozone, a common constituent of urban smogs. Exposure assessment is critical to understanding the risks posed by exposure to hazards in the environment.

Hazard identification is the toxicology (animal and human) and epidemiology that provides the biological information on the response of an organism to the exposure (dose) of a toxic substance. Dose-responses can be threshold, linear non-threshold or hormetic [4 – 6]. The responses measured can range from acute to chronic toxicity.

This review will focus only on chemical hazards, although "hazard" may be defined as being either chemical, physical (e.g. radiation) or biological in nature. Furthermore, although it is recognized that the risk assessment

of militarily relevant toxicants in the field may involve chemical exposures and assumptions that are unique to military science, we will focus on how different agencies assess them in occupational and environmental scenarios; their different approaches being instructive to the science as a whole. This review is not exhaustive in breadth and is only intended to provide an overview of how chemical mixtures toxicology assessment is being pursued.

5.4 DIFFERENT APPROACHES TO MIXTURE ASSESSMENT

Several techniques have been devised to assess the toxicity of chemical mixtures. An excellent review of these different methods can be found in a recent Agency for Toxic Substances and Disease Registry (ATSDR) guidance document [2]. Each of the assessment methods mentioned in the ATSDR document are briefly described below.

One of the simplest ways to assess mixtures is the Hazard Index (HI), which uses dose additivity. For each component of the mixture, the exposure level is divided by a defined level of exposure that causes a toxicological effect (e.g. Threshold Limit Value, TLV). This ratio is calculated for all components of a mixture and summed to define the HI for the mixture. As the HI approaches unity, there may be concern for effects from the mixture. The HI method does not consider interactions between components of the mixture.

A modification of the HI is the Target-organ Toxicity Dose (TTD) method which allows for assessment of chemical mixtures where the components do not all have the same critical toxic effect. If components of a mixture have effects on different systems in the body, the TTD accounts for this when the component level reaches a threshold where the critical effect will occur. The TTD is calculated for each endpoint of concern and then used to estimate endpoint-specific hazard indexes. When any of these HI approach or exceed unity, the potential for toxic effects from exposure to the mixture is increased.

The Weight-of-Evidence Modification to HI (WOE) accounts for interactions by using weight of evidence for interactions among pairs of components of the mixture. Each possible pair of chemicals is evaluated in order to make binary weight-of-evidence determinations for the effect of each chemical on the toxicity of each other chemical. In the WOE modification, changes in proportions of mixture components are not accounted for and the model assumes that all chemical interactions are only binary in nature (A can affect B and B affect A but C does not affect these interactions).

Toxic Equivalency and Relative Potency compare mixture components against a component that has been sufficiently well investigated with respect to health information. This technique assumes dose additivity, and the assessment is expressed as toxic "equivalents" of a known component (usually the most toxic) of the mixture. Environmental samples containing dibenzo(p)dioxins are perhaps the best known examples of being assessed using the toxic equivalency approach; their potential toxicity often being expressed as "2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalents".

Two other methods that may be used for carcinogenic endpoints are the Total Cancer Risk and the Integral Search System (ISS). Total Cancer Risk assumes the response or risk for a mixture is the sum of the risks for cancer for all of the components based on the dose and potency parameters for each component. ISS uses data for binary mixtures to predict the hazard of exposure to mixtures of three or more chemicals. ISS does not include exposure information or dose as part of the assessment.

Physiologically-based pharmacokinetic modeling (PBPK) uses computer modeling of biological information to assess the potential for interactions between chemicals in biological systems. PBPK modeling can be used to

predict effects from co-exposure for different scenarios. These scenarios may represent changes in components in the mixture or changes in exposure concentrations.

A variety of different approaches has been utilized by various national and international organizations to assess how chemical mixtures potentially affect human health with respect to occupational and environmental exposures. An examination of these methods, the assumptions upon which they rest and their subsequent shortcomings, should provide insight as to how to approach the hazard assessment of mixtures in a military operational scenario.

5.5 OCCUPATIONAL EXPOSURE ASSESSMENT

Occupational exposure assessment generally examines higher dose/exposure levels than an ambient or environmental assessment. This is because the occupational situation generally occurs for a specified time period (e.g. 8 hours/day; 5 days/week) in a specific environment. Different countries and political/economic blocks have developed ways of evaluating occupational exposures to mixtures. Many of these are simply harmonized labeling or classification conventions but they could be used for a cursory assessment of chemical mixtures.

Canada, at the federal level, has established the Workplace Hazardous Materials Information System (WHMIS) which uses the "Controlled Products Regulations" (CPR) legislation to classify chemicals and mixtures based on different endpoint criteria (CPR 46 to 65). For each compound, a WHMIS classification is established. For mixtures, a toxicological evaluation is carried out by taking the LD_{50} or LC_{50} of every ingredient present at a concentration of one per cent or more. If this information is known, the LD_{50} or LC_{50} of the mixture is determined by calculation of a proportional representation of the constituent compounds. If the LD_{50} or LC_{50} of one or more of the ingredients is not known, the LD_{50} or LC_{50} of the mixture is equal to the LD_{50} or LC_{50} of the most acutely lethal ingredient that is present in the mixture at a concentration of one percent or more and for which LD_{50} or LC_{50} data is available. This legislation relies heavily on Test Guidelines from the Organization for Economic Co-operation and Development (OECD) for specific endpoints assessment. See [7] for an overview and links.

In the USA there are a variety of occupational regulatory agencies that vary in their approach to risk assessment. The Occupational Safety and Health Administration (OSHA) sets a permissible exposure limit (PEL) for occupational exposures. OSHA recommends a hazard index approach similar to the American Conference for Governmental Industrial Hygienists (ACGIH; see below) where the sum of the ratios must not exceed unity (i.e. one). The approach is not restricted to substances that have similar effects. OSHA places the responsibility for evaluation on the manufacturer, importer or employer and if a mixture has been tested as a whole, the results are used to determine if the mixture is hazardous. If the mixture has not been tested as a whole, it is assumed that the mixture will present the same health hazards as do the components which comprise one percent (by weight or volume) or greater of the mixture. The exception to this is if a component is a carcinogen which comprises 0.1% or greater of the mixture. In this case, the mixture will be considered as a carcinogenic hazard. The potential physical hazard presented by a mixture must be addressed as well. If a component present in a mixture in concentrations of less than one percent (<0.1% for carcinogens) could be released in concentrations that could exceed an OSHA PEL or ACGIH Threshold Limit Value (TLV), or could present a health risk at those concentrations, the mixture assumes the same hazard as that component [8].

The National Institutes for Occupational Safety and Health (NIOSH) in the USA has recognized the need for further development of assessment methodologies with respect to mixture toxicology [9].

The strategies for non-cancer and cancer-causing effects from exposure to mixtures being proposed by the ATSDR in the USA are similar (See Fig 2 and 3; [2]). The executive summary in this document [2] provides a clear explanation on how the ATSDR proposes to assess exposures to chemical mixtures … "Exposure data and toxicological information on the mixture of concern (or a similar mixture) are the preferred basis for an assessment. If available, toxicological information on mixtures of concern are reviewed and evaluated from ATSDR documents, including interaction profiles and toxicological profiles. If specific ATSDR documents or comparable documents from other agencies are not available, or do not provide Minimal Risk Levels (MRLs) or comparable health guideline values for the mixture or guidance regarding a health assessment approach, and if suitable whole mixture studies are not available, a components-based approach is undertaken. The components-based approach focuses on mixture components which are present at toxicologically significant exposure levels, based on estimated exposures and relevant health guideline values. Linked physiologically-based pharmacokinetic/pharmacodynamic (PBPK/PD) models for two or more components, if available, may be used to predict the potential for interactions, or possibly for non-cancer or cancer health effects from the mixture. The hazard index method is used to screen for non-cancer health hazards from potential additivity of the components. Cancer risks for the components are summed to screen for health hazards from potential additivity of carcinogenic effects. A weight-of-evidence method is used to evaluate the potential impact of interactions on non-cancer and cancer health effects." [2]. ATSDR reviews both occupational and environmental exposure.

In the European Union, a committee was established to provide advice on chemical exposure. The Scientific Committee on Occupational Exposure Limits (SCOEL) [10] started as an informal group of scientists formed to provide advice to the European Commission and eventually was formalized in 1995. Experts in chemistry, toxicology, epidemiology, occupational medicine and industrial hygiene are appointed to this group. The SCOEL makes recommendations on health based Occupational Exposure Limits (OEL) which may include eight-hour time weighted averages (TWA), short-term / excursion limits (STEL) and biological limits. The SCOEL evaluates all available data and then proposes a recommendation which provides the scientific basis for exposure limits included in legislation. Member States then utilize this information to establish exposure values. It is not explicitly clear how SCOEL assesses chemical mixtures or if there is a formalized process.

In Australia, the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) [11] and the National Occupational Health and Safety Commission [12] are responsible for providing guidance on assessment of chemical mixtures.

Other organizations have also developed, or started to develop, guidelines and recommendations for exposures to chemical mixtures. Perhaps the most germane to this paper is the USA National Academies of Science current project evaluating Toxic Industrial Chemicals and chemical warfare agents [13] (see Review of the Army's Technical Guidance Documents on Assessing Toxicological Risks from Exposures to Chemicals). This is a review of the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 230 entitled "Chemical Exposure Guidelines for Deployed Military Personnel".

The American Conference of Governmental Industrial Hygienists in the USA utilizes an additivity approach for assessing the hazards posed by mixtures (hazard index approach) [14]. The substances must act on the same organ system. The ratio (C/T) of the exposure concentration (C) to the TLV (T) for each substance is summed. If the sum exceeds one, the TLV for the mixture has been exceeded. This approach is not used if the effects are on different systems. If the primary effects of the different components are not additive but are independent, e.g. when different organs are affected, the TLV is considered to be exceeded when at least one component C/T ratio in the mixture exceeds one. When a number of harmful dusts, fumes, vapours, or gases are released,

ACGIH indicates that the only feasible approach may be to measure a single substance in order to evaluate the hazard. The threshold limit for this substance should be reduced by some factor (the magnitude of which takes into account the number, toxicity, and relative amounts of the other components typically present).

The United Nations Economic Commission for Europe (UNECE) has set forth to establish a globally harmonised system (GHS) of hazard classification and labeling for the safe use of chemicals in the workplace and the environment in general [15]. The hazard classification process refers principally to the hazards arising from the intrinsic properties of chemical elements and compounds and mixtures of these elements (Parts 2 and 3 [15]). Substances are classified according to their health, environmental and physical hazards. Mixtures are handled in the following manner: a) classification of the mixture will be based on test data for the complete mixture, if available, or b) bridging principles (see Section 3.1.3.5 in Part 3 [15]) will be used to determine classification of the mixture. For health and environmental classification, if neither "a" or "b" are sufficient, then a method is agreed upon based on known information to classify the mixture. Specific considerations for classifying mixtures have been identified but a significant level of responsibility rests with the reviewer/ evaluator in the final classification.

The Organization for Economic Co-operation and Development established an advisory group to examine harmonisation of classification and labeling of compounds and this group in turn, established an expert group to study mixtures. This program does not require testing of chemicals but makes use of existing data. The criteria are laid out in detail in [16]. Briefly, the process of classification is as follows: a) where data are available for the complete mixture, classification will be based on that data; b) where data are not available for the complete mixture, bridging principles are to be considered (see [16] for detailed explanation of principles); c) if information is not sufficient to allow for bridging principles to be applied, then agreed methods for estimating the individual hazards based on known information will be applied. Part 3 of the OECD monograph [16] provides details on the classification of chemical mixtures which cause acute toxicity, skin and eye corrosion/irritation, respiratory or skin sensitisation, germ cell mutagenicity, carcinogenicity, reproductive toxicity, or specific target organ systemic toxicity.

5.6 ENVIRONMENTAL EXPOSURE ASSESSMENT

Ambient or environmental exposure to pollutants means non-occupational exposures. These are generally at lower exposure concentrations for longer periods of time (e.g. 24 hours per day for 7 days per week). They encompass indoor and outdoor environments and may even include microenvironments such as inside a vehicle.

In Canada, environmental exposure assessments for toxics are carried out under the Canadian Environmental Protection Act (CEPA). With respect to mixtures, the approach as to whether a chemical substance is considered "toxic" under CEPA is dependent upon the nature of available data on the mixture. As with occupational exposures, at times the mixture composition, exposure levels and toxic effects may be well characterized. Generally, this is not so and the assessment is usually on a case-by-case basis. For those cases in which information is available the approach to assess whether or not it is "toxic" under CEPA is similar to that for single "threshold" or "non-threshold toxicants". See Health Canada [17] for an explanation of "toxic", "threshold" and "non-threshold" as defined by CEPA.

Assessing whether simple mixtures are "toxic" under CEPA can be based on the effects of either some or all of the components present in the mixture. For those cases in which the components in the simple mixture have similar effects due to similar modes of action, and there is little indication for interaction between components, effects are generally considered to be additive.

One approach for assessing "threshold toxicants" involves determining the total daily intake of the mixture as toxic equivalents (summing of the concentrations of individual compounds multiplied by the potency of that substance relative to that of the reference [generally most potent] substance). This composite measure is then compared to a Tolerable Daily Intake (TDI) for the reference substance, derived as for "threshold toxicants". A hazard index (HI) approach is used for simple mixtures where the components are classified as unlikely to be carcinogenic, to unclassifiable with respect to carcinogenicity in humans and for which the mechanisms of toxicity for the critical effect are similar. The HI is derived in a manner similar to that previously mentioned but substitutes 1/TDI for the relative potency factor. If the numerical value of the hazard index exceeds one, the simple mixture is considered to be "toxic" under CEPA. In cases where the simple mixture contains a high proportion of substances classified as carcinogenic or probably carcinogenic to humans, or as a human germ cell mutagen or probable mutagen, the mixture as a whole may be considered "toxic" under CEPA. Such a determination is based on consideration of factors such as the extent of characterization of the chemical composition and toxicological effects of the simple mixture and the proportion of the total mixture that is composed of components classified as (probable) carcinogens/mutagens. For simple mixtures considered to be toxic owing to the classification of a major proportion of components as carcinogens/mutagens, where possible, the estimated daily intake of the components by the general population or concentrations in relevant environmental media are compared to quantitative estimates of carcinogenic or mutagenic potency (Exposure/ Potency Index or EPI) to characterize risk and provide guidance in establishing priorities for further action following assessment of toxicity under the Act [17].

The USA Environmental Protection Agency (USEPA) has led the charge in the analysis of effects of mixtures in the general population (non-occupational exposure) [1]. This document has been recently reviewed and updated [3]. The method of assessment for a chemical mixture ultimately depends on the quality of data available to determine if the mixture will be assessed as a whole or as components. For any chemical mixture, all possible paths to the final summary should be examined as a relevant assessment method (See Figure 2-1 in [3]). The USEPA stresses the importance not only of the health effects and interactions information available for assessment but also of the quality of the exposure data. Some of the essentials required to quantify the exposure to chemical mixtures include: concentration of the mixture at the point of contact; duration and frequency of exposure; accuracy and reliability of the measurement techniques used; determination of whether all components have been identified; bioavailability of the mixture for the medium and route of exposure. The USEPA is currently working on developing cumulative risk assessment guidelines. The external review draft of the framework for these guidelines is available [18]. The EPA defines cumulative risk as the combined risks from aggregate exposures to multiple agents or stressors.

The European Union (EU) recently announced a "European Environment and Health Strategy" [19]. Part of this strategy will be science-based and will examine the interactions of pollutants in the environment and the impact on public health. "Traditionally, environmental assessments and policy action have focused on single pollutants in single environmental compartments, such as air, water or soil. There is, however, a strong need to look into how different pollutants react together. We need to better understand how pollutants move in the environment and how we come in contact with them – through air, water, food, and consumer products. We also need to understand how the human body reacts, over a period of time, to continuous exposure to different pollutants, interacting between each other, often at a low level." [19]

5.7 CURRENT AND EMERGING ISSUES

A number of problems continue to plague the accurate assessment of chemical mixtures. While many of the individual components of a mixture may be adequately documented, there is usually a significant lack of toxicity data on the complete mixture. In addition complex chemical interactions within the mixture may

result in unforeseen by-products which have an inherent, also unforeseen, toxicity. The lack of adequate exposure information also complicates the assessment (poor dosimetry). Finally, another factor that is not usually discussed and is highly relevant is not only the effect of stress on exposure and resultant toxicity, but also that of exposure-induced stress. In their study, Friedman and Lawrence [20] discussed the effect of chemical, physical and psychological stress on health status from exposures.

5.8 WHAT'S ON THE HORIZON?

The major problems which keep coming up are the lack of toxicology data and valid exposure data for mixtures. The explosion of genomics and proteomics could prove to be a goldmine with respect to large scale testing of complex mixtures at the molecular biology level of investigation. While providing a wealth of information, these techniques are not well developed enough yet to provide rapid assessments. PBPK modeling is also progressing to the point where it is starting to provide invaluable information to scientists, evaluators and regulators investigating the effects of complex chemical mixtures [21 – 23]. Small, sensitive dosimeters capable of providing accurate and rapid chemical exposure data are being developed which will provide exposure information that is lacking or not representative of exposure at the level of entry into the body. Multimedia exposure assessment strategies have developed significantly over the last 10 years and coupled with realistic exposure data have rapidly expanded the ability of the toxicologist/evaluator to assess the dose presented to biological systems.

5.9 CONCLUSIONS

The issue of understanding the effects from exposure to chemical mixtures is not trivial. In many ways this part of toxicology is in its infancy. It also presents significant problems to evaluators/regulators as each chemical mixture can be geographically distinct in the environment (e.g. air pollutants). Different government and non-government bodies have developed detailed strategies to assess what levels may be considered safe but there are several limitations to these strategies. Many do not account for interactions either at a chemical or biological level, or are only effective if there is sufficient toxicological and exposure information on the whole mixture. If this information is not present, the task of assessing the mixture becomes filled with uncertainty. Another problem is that it is assumed that the exposure and subsequent health effects are from exposure to the native form of the mixture and not to the mixture after it has been physically altered (e.g. combustion) and mixed with other compounds or mixtures. There is significant effort being applied to the assessment of exposures to mixtures whether the exposure is occupational or environmental. The information, guidelines and regulations coming from these efforts can act as a guide to assist in developing methodologies to assess risk to troops in different chemical environments. Developing and validating these models and algorithms would be a significant undertaking.

The rapid expansion of techniques being exploited by toxicologists will provide data in the future that will greatly assist in the assessment of exposures to mixtures. While some of these techniques may not speed up the assessment process, they will bring a volume of data to the assessment arena that has not been seen before. The examination of whole complex mixtures will benefit from the advancement of genomics, proteomics, and PBPK modeling but the process of assessment itself will become more complex and difficult.

5.10 ACKNOWLEDGMENTS

The author would like to acknowledge Cory Vair for his assistance in formatting and editing of the drafts of the document.

Table 5-1: Links to Relevant Organizations

Agency for Toxic Substances and Disease Registry (ATSDR); United States of America: http://www.atsdr.cdc.gov/
American Conference of Governmental and Industrial Hygienists (ACGIH); United States of America: http://www.acgih.org/
Canadian Environmental Protection Act (CEPA); Canada: http://www.ec.gc.ca/CEPARegistry/default.cfm
Environmental Protection Agency (USEPA); United States of America: http://www.epa.gov/
European Environment and Health Strategy (EEHS); European Union: http://europa.eu.int/comm/press_room/presspacks/health/pp_health_en.htm
Globally Harmonized System of Classification and Labelling of Chemicals (GHS); United Nations Economic Commission for Europe: http://www.unece.org/trans/danger/publi/ghs/officialtext.html
National Academies of Science; United States of America: http://www.nationalacademies.org/
National Industrial Chemicals Notification and Assessment Scheme (NICNAS); Australia: http://www.nicnas.gov.au/
National Institutes for Occupational Safety and Health (NIOSH); United States of America: http://www.cdc.gov/niosh/homepage.html
National Occupational Health and Safety Commission (NOHSC); Australia: http://www.nohsc.gov.au/
Occupational Safety and Health Administration (OSHA); United States of America: http://www.osha.gov/
Organisation for Economic Co-operation and Development (OECD): http://www.oecd.org/home/
Scientific Committee on Occupational Exposure Limits (SCOEL); European Union: http://europe.eu.int/comm/employment_social/health_safety/areas/oels_en.htm
Workplace Hazard Material Information System (WHMIS); Canada: http://www.hc-sc.gc.ca/hecs-sesc/whmis/index.htm

5.11 REFERENCES

[1] United States Environmental Protection Agency. *Guidelines for the Health Risk Assessment of Chemical Mixtures* (EPA/630/R-98/002). Washington, D.C.; United States Environmental Protection Agency, 1986.

[2] Agency for Toxic Substances and Disease Registry. *Guidance Manual for the Assessment of Joint Toxic Action of Chemical Mixtures* – Draft for Public Comment. Washington, D.C.; United States Department of Health and Human Services, 2001.

[3] United States Environmental Protection Agency. *Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures.* (EPA/630/R-00/002). Washington, D.C.; United States Environmental Protection Agency, 2000.

[4] Calabrese, E.J. and Baldwin, L.A. Toxicology rethinks its central belief. *Nature* 2003; 421:691-692.

[5] Calabrese, E.J. and Baldwin, L.A. Applications of hormesis in toxicology, risk assessment and chemotherapeutics. *Trends Pharmacol Sci* 2002; 23(7):331-337.

[6] Jayjock, M.A. and Lewis, P.G. Implications of hormesis for industrial hygiene. *Hum Exp Toxicol* 2002; 21(7):385-389.

[7] Health Canada. Workplace Hazard Material Information System (WHMIS). Online at: http://www.hcsc.gc.ca/hecs-sesc/whmis/index.htm (Last accessed 1 February 2007).

[8] Occupational Safety and Health Administration. Occupational Safety and Health Standards. OSHA Regulations (Standards – 29 CFR) Air contaminants. – 1910.1000; Hazard Communication – 1910.1200) Online at: http://www.osha.gov/ (Last accessed 5 June 2007).

[9] Department of Health and Human Services. *Exposure Assessment Methods: Research Needs and Priorities*. (DHHS [NIOSH] Publication No. 2002-126). Washington, D.C. Department of Health and Human Services, 2002.

[10] European Commission. Scientific Committee on Occupational Exposure Limits (SCOEL). Online at: http://ec.europa.eu/employment_social/health_safety/docs/oel_neurotoxicity_en.pdf. (Last accessed 5 June 2007).

[11] Department of Health and Ageing, Australian Government. National Industrial Chemicals Notification and Assessment Scheme (NICNAS). Online at: http://www.nicnas.gov.au/. (Last accessed 5 June 2007).

[12] Australian National Occupational Health and Safety Commission (NOHSC). Online at: http://www.ascc.gov.au/. (Last accessed 5 June 2007).

[13] National Academy of Sciences. The National Academies. Online at: http://www.nationalacademies.org/. (Last accessed 1 February 2007).

[14] American Conference of Governmental Industrial Hygienists. *2003 TLVs and BEIs*. Cincinnati, Ohio; ACGIH, Inc., 2003:224.

[15] United Nations Economic Commission for Europe. *The Globally Harmonized System of Classification and Labelling of Chemicals*. Online at: http://www.unece.org/trans/danger/publi/ghs/ghs_rev00/00files_e.html. (Last accessed 1 February 2007).

[16] Organization for Economic Co-operation and Development. *Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures*. OECD Series on Testing and Assessment Number 33. Paris, France; Organization for Economic Co-operation and Development, 2001.

[17] Health Canada. *Human Health Risk Assessment for Priority Substances*. Ottawa, Canada; Canada Communication Group – Publishing, 1994.

[18] United States Environmental Protection Agency. *Framework for Cumulative Risk Assessment – External Review* DRAFT. (EPA/630/P-02/001A). Washington, D.C.; United States Environmental Protection Agency, 2002.

[19] European Commission. *Environment and Health; the European Commission Launches a Strategy to Reduce Diseases Linked to Environmental Factors*. (IP/03/823). Brussels, Belgium; European Commission, 2003.

[20] Friedman, E.M. and Lawrence, D.A. Environmental stress mediates changes in neuroimmunological interactions. *Toxicol Sci* 2002; 67(1):4-10.

[21] Conolly, R.B. Biologically motivated quantitative models and the mixture toxicity problem. *Toxicol Sci* 2001; 63 (1):1-2.

[22] Haddad, S., Beliveau, M., Tardif, R. and Krishnan, K. A PBPK modeling-based approach to account for interactions in the health risk assessment of chemical mixtures. *Toxicol Sci* 2001; 63(1):125-131.

[23] Haddad, S., Charest-Tardif, G., Tardif, R. and Krishnan, K. Validation of a physiological modeling framework for simulating the toxicokinetics of chemicals in mixtures. Toxicol. *Appl Pharmacol* 2000; 167(3):199-209.

Chapter 6 – NEUROBEHAVIORAL TOXICITY ASSESSMENTS

by

Karl Friedl, Stephen Grate, and Susan Proctor
U.S. Army Research Institute of Environmental Medicine, Natick, MA, U.S.A.

This chapter formed the basis of a paper subsequently published in a special volume of Archives of Clinical Neuropsychology 2007; 22 (supplement1): 7 – 14 summarizing U.S. Department of Defense applications of automated neuropsychological testing.

6.1 ABSTRACT

Information on the mental status of Soldiers is vital to their management in future deployments to prevent acute performance deficits and post-deployment health consequences such as chronic multisymptom illnesses and neurodegenerative diseases. The military needs a parsimonious set of reliable neuropsychological tests that:

1) Provide early detection of individual impairment; and

2) Predict occupational and deployment health risks.

Testing must characterize cognitive lapses and mood changes in healthy individuals faced with relevant operational stressors, including chemical exposures and interactions with other stressors, and should be based on understanding effects of final common pathways in brain physiology (e.g. hypo- or hyperglycemia, and hypoxia, oxidative stress, and inflammation). Stressors may affect a common hierarchy of deficits and batteries could be reduced to a few tests such as simple reaction time, matching-to-sample, running memory, math processing, and code substitution; alternatively, these tests together might provide a differential diagnosis. The ultimate goal of unobtrusive real-time mental status monitoring may one day provide the most sensitive indication of a neurochemical exposure.

6.2 INTRODUCTION

The Soldier is the acknowledged centerpiece of the Army's warfighting system, and military success will largely depend on the mental status of these individuals. The complexity, speed, and lethality of modern warfare means that even small mental lapses may have catastrophic consequences. Judgment and other forms of decision-making, mood and cooperation, psychomotor performance, and cognitive status are all critical elements of Soldier performance. In the military, these elements have been traditionally evaluated by observation or, after the fact, as net outcomes such as task or mission completion. Thus, rigorously specified neuropsychological testing methods are needed to fill a vital technological gap in Soldier health and performance research [1, 2]. A very important part of this effort is to explore the neuropsychological methods in militarily relevant conditions to extend our understanding of relevant functional domains and how well they correspond to modes of testing. The Automated Neuropsychological Assessment Metric (ANAM) was started by Dr. Fred Hegge as a means to provide standardized and valid testing for a wide variety of military applications [3, 4]. The intent is to serve as a plug-and-play toolkit of tests for research and for practical application (Table 6-1). This paper discusses the military research needs for ANAM test batteries in terms of neurotoxicity assessments and relevant neurophysiological studies.

Table 6-1: Potential Military Applications of ANAM

Objective	Examples of Potential Applications
Health Applications • Baseline status of new recruits • Periodic health status assessment • Occupational hazards monitoring • Post-deployment health monitoring • Safety evaluations of new materiel	• Recruit Assessment • Comprehensive Health Care System II (CHCSII) • Preventive medicine investigative tool Program (RAP) • Early diagnostic tool in redeployment screening • FDA-approved testing standard
Performance Applications • Fitness for duty and return-to-duty status • Real-time soldier status monitoring • Human factors evaluations of new materiel and combat concepts	• Pre-mission screening tool • Embedded task testing in future systems • MANPRINT

6.3 APPLICATIONS OF NEUROPSYCHOLOGICAL TESTING

6.3.1 Health Assessments

Currently there is no practical method to establish the baseline mental status of new recruits. The only information recorded that may help in the evaluation of future neurotoxicity issues is from the conventional medical history and general aptitude testing from the Armed Forces Qualification Test. A new initiative to collect more detailed health and psychological status within the first week of military service, the Recruit Assessment Program (RAP), still only provides self-report questionnaire data [5]. An efficient session, and possibly repeat sessions, with a brief ANAM test battery might provide exceptionally valuable information. This test battery would have to be well validated for reliability and reproducibility and, most importantly, demonstrate value in detecting meaningful changes in health and performance status for early disease states or non-obvious changes in militarily relevant performance that might be traceable to chemical exposures. These dangers could be resolved sooner if a sensitive test methodology for early detection existed. A key platform for this future standardized testing and data repository would be the Comprehensive Health Care System II (CHCSII) database [6].

6.3.2 Readiness Status Assessments

Taken a step further into the future, periodic neuropsychological testing of all military members might be invaluable to readiness status assessments, early identification of illness, and detection of unanticipated occupational or deployment health risks. This would provide an opportunity to detect neurotoxic influences and other neurological susceptibilities in individuals before deployment. Currently, the Army conducts physical fitness testing and weighs all members twice a year to ensure individual physical readiness.

In a technologically sophisticated future force, it will be important to commit at least equal emphasis on cognitive readiness, perhaps including Nintendo-like game tests or timed puzzle-solving challenges that will have been derived from current investigations using automated neuropsychological testing. Like remedial physical training for those not meeting physical fitness criteria, further evaluation and remedial training to build cognitive resilience or neurophysiologically-based skills could be developed. This new emphasis on cognitive readiness will likely converge with physical training standards as we learn more through new Army research initiatives on the neurobiology of exercise and neuropsychological testing in physically active Soldiers.

6.3.3 Materiel Safety Evaluations

Materiel safety evaluations require a well-standardized test methodology. The tests in such a battery must have clearly demonstrated thresholds or definitions of impaired neuropsychological performance. This test capability (i.e. norms and thresholds) is important for generation of valid test data to support approval of new drugs and other medical materiel. The Army needs to go a step beyond traditional FDA approvals, to include testing of new products in operationally relevant environments to ensure that there are no important interactions between the product and key operational stressors. Under the Army's MANPRINT process of new system evaluation, even non-medical materiel concepts that do not require FDA approval are required to consider effects on human health and performance. MANPRINT requires consideration of human factors and medical safety in the design of new equipment at all stages of the development process [7]. Therefore, a relevant test battery that provides valid data in messy field conditions will be especially important. The neurobehavioral testing methods that have been well developed for neurotoxicological assessments [8, 9] provide a strong basis for field investigations of unforeseen interactions, such as the current initiatives to develop these tools using permethrin and JP8, the intended universal military fuel, as the initial focus.

6.3.4 Relevance to Military Performance Outcomes

In addition to standardization and validation of testing methods, research is needed to clarify the measurements that correspond to militarily relevant performance outcomes. This was a key objective of the Office of Military Psychological Assessment Test (OMPAT) program, which initiated development of the ANAM and other electronic testing softwares such as the MicroSaint simulation software to study crew interactions. The OMPAT program was initiated in 1984 to assess the impact of operationally employed drugs on normal performance. The program was funded from the medical chemical defense program, with specific interest in nerve agent prophylaxis. However, the problem continues to defy research attempts.

6.3.5 Performance Assessments

Future Soldier concepts include technologically enabled individuals operating in "net-centric" teams with unprecedented access to information, decision-assist technologies that will suggest solutions to forecasted problems, multi-sensory inputs ranging from tactile feedback to 3-D audio spatial "displays," and instant fingertip or voice access to unmanned aerial vehicles and other remote robotic weapons and intelligence systems, and other equipment and adornments. The future Soldier will be an extraordinarily powerful and lethal system, and decision-making ability of the human element will be of critical importance. Commanders will demand assurances that Soldiers are mentally fit for duty and may rely on monitoring systems that will indicate when human failure is imminent. The new technologies will add to the neuropsychological burden of the Soldier, but also provide more opportunities to unobtrusively monitor performance status through physiological measures; for example, emotional status estimated from voice stress analysis via existing communications systems [10] to task performance testing embedded in routine check-in and calibration

procedures. Reliability of the mental status assessments of the Soldier will be even more important in any applications where the assessment system can automatically take control away from the Soldier in the loop. This concept of machine decision assist is the basis of several current DoD programs that range from balancing workload and decision making between team members based on the cognitive status assessment of each individual to systems that would take away controls of an aircraft if the pilot appears to be impaired, either through physiological measurements or illogical responses [11].

6.3.6 Warfighter Physiological Status Monitoring Systems

In some settings, Soldiers may be operating in remote areas away from a main force, or they may be out of sight of their team members during operations in urban terrain, or while fully encapsulated in protective clothing and masks. In these cases, it could be important to have objective information on the cognitive status of an individual or a team which cannot be more directly observed or monitored. Ideally, the monitoring system would involve passive and continuous assessments. This is the concept behind Warfighter Physiological Status Monitoring (WPSM), a system of wear-and-forget non-invasive physiological monitors that will use a combination of sensor signals to determine how a Soldier is doing, when the Soldier has been injured, and when the Soldier is headed for trouble in terms of injury consequences (e.g. overheating, dehydration, overexertion) or in terms of performance lapses (from e.g. toxic chemical exposures, fatigue, psychological stress, information overload) [12]. Predicting impending problems from physiological signals is difficult because of the compensatory mechanisms that sustain an individual in the face of external challenges until compensation is no longer possible (i.e. the distinction between the “amber” warning of impending trouble and the “red alert” when the Soldier is already a casualty). Neuropsychological testing in experimental settings is likewise affected by subjects temporarily rallying to complete a test even when they may be extremely fatigued or challenged in some other way. The earliest form of a WPSM approach to neurocognitive assessments is likely to use extremes of the physiological range of measures such as very low or high blood glucose levels (from minimally invasive sub-dermal probes) and recent sleep history (from wrist-worn accelerometry) to predict likely performance lapses. Whatever system is employed will have been developed against a reference standard of automated neuropsychological testing. It is also likely that for some critical assessments of cognitive status, relatively unobtrusive testing will be built into regularly scheduled tasks or games that Soldiers will perform using computers that every Soldier will be wearing.

6.4 HYPOTHESES CONCERNING OPERATIONAL STRESSORS AND TEST PERFORMANCE

6.4.1 Common Brain Physiology Mechanisms Produced by Military Stressors

The nature of the deficits that occur in various physiological states has not been well mapped. For many key stressors there appears to be a general trend to preserve core survival functions at the cost of higher cortical functions (i.e. loss of function progresses in a rostral-caudal direction), suggesting that for some applications, a test battery could be devised to specifically test this progression of impairment. This appears to be the case for hypoglycemia, with marked changes in cognitive testing occurring below 3 nmol/L; memory is one of the first tests affected [13]. This is also the case with acute hypoxia, with an average 25% reduction in short-term memory during ascent from sea level to over 14,000 feet altitude, corresponding to a reduction from 96 to 40 torr in arterial partial pressure of oxygen [14]. Likewise, cold exposure (e.g. 1 hour or more at 4ºC, or immersion in water at 10ºC) produces reductions in match-to-sample testing more consistently and with greater sensitivity than any other neuropsychological test [15, 16]. More remarkable in these tests is the finding that tyrosine ingestion can reverse the deficit [17]. Along with more definitive data from brain

microdialysis studies in rats, this suggests that catecholamine synthesis in the brain plays a role and that increased substrate availability may be all it takes to counter deficits that are produced by this mechanism of stress-induced catecholamine reduction [17]. In each of these three stressors (neuroglycopenia, hypoxia, and cold), working memory is one of the first functional axes affected, followed by tests in other domains such as attentiveness. This suggests that for some monitoring applications, relatively simple test batteries could be devised that might provide a generalizable assessment of acute performance status.

6.4.2 Other Stressors

6.4.2.1 Chronic Psychological Stress

Other stressors, especially involving longer exposures (e.g. >24 hours), appear to produce more specific and unique functional deficits. Some of these have been attributed to specific mechanisms such as the link between chronic cortisol exposure, shrinkage of the hippocampal volume, and effects on memory functions [18]. Chronic cortisol exposure could result from a variety of stressors, although chronic anxiety, severe trauma, and other psychological factors are probably the most important.

6.4.2.2 Head Impact

Although head impact can be a focal insult affecting a specific region of the brain, concussion has generalized effects on the brain, and Warden, Bleiberg, and colleagues [19, 20] suggested that global speed of processing reflected in a simple reaction time might even provide a practical single test of impairment and return-to-duty status. This is being further investigated in cadet boxing and parachute-landing models in the military, and has already provided pioneering studies in baseline and repeated testing methods.

6.4.2.3 Sleep Deprivation

Sleep deprivation and short-term interventions have been well studied by the military. This provides a different model of neuropsychological impairments which may be traceable to observed hypometabolic responses in specific regions of the brain. A greater than 50% reduction in “throughput” (primarily a reduction in speed) on several neuropsychological tests at 48 hours of sleep deprivation is reversible for 4 – 6 hours with 600 mg of caffeine [21]. Memory tests are not very sensitive to the effects of this stressor, especially compared to tests of attentiveness such as the psychomotor vigilance test (PVT) [22]. The PVT should provide direct comparisons to ANAM tests with other stressors.

6.4.2.4 Commonalities in Test Outcomes

Related mechanisms involving oxidative stress, inflammation, and excitatory neurotransmitters may be common to many of these other stressors. Regardless of the stressor, there appears to be a common behavioral strategy to handle impairments. In a variety of tests that includes hypoglycemia, concussion, and sleep deprivation, the overall speed of mental processing is typically sacrificed to preserve accuracy [13, 20, 21]. If this rule can be reliably established for certain test applications, an index based on accuracy and speed might form the basis of a measure of mood status and motivation to perform well.

6.4.3 Neurotoxic Exposures

Neuropsychological testing relevant to military applications is most advanced in the area of neurotoxicology [8, 9, 23, 24]. Various classes of chemicals have been characterized for their highly specific regional and

functional effects. In some cases, neuropsychological effects of heavy metals, solvents, organophosphates, and various agricultural and industrial chemicals provide the primary classification of biological effects, where physiological and anatomical lesions may be much more difficult to measure. ANAM testing methods could be further advanced with validation against the better-established neurotoxicology models [25]. Current Army initiatives include testing effects of permethrin and JP8, following up on other human and animal studies demonstrating neurocognitive deficits [26, 27]. Mood changes are among the first and most consistent changes with neurotoxicants. The influence of mood state and other additional psychological and environmental stressors on neurotoxicological responses in a military deployment needs to be carefully studied to make practical field tools useful. Table 6-2 lists some of the key priorities for development of these health monitoring methods.

Table 6-2: Examples of Potential Neurotoxicants Relevant to the Military and Occupational and Post-deployment Health Applications

- Blast overpressure and combustion gases inside defeated military vehicles and craft*
- Petroleum products*
- Chemical Agent Resistant Coatings, paints, solvents, painting, decontamination
- Combustion products from burning garbage dumps
- Smokes and obscurants
- Insecticides (e.g. permethrin and DEET)
- Agricultural chemicals, especially those banned as harmful in the U.S. (e.g. dieldrin)
- Air quality in closed compartments (e.g. submarines)
- Heavy metals used as armor and armor penetrators (e.g. depleted uranium)*
- Industrial chemical contaminants of high toxicity (e.g. methyl mercury)*
- Mixtures of industrial wastes (possibly including radiological waste)*
- Interactions of toxic chemicals with electromagnetic radiation (e.g. radar systems)
- Interactions of toxic industrial/agricultural chemicals with operational factors (e.g. heat, psychological stress, dust)
- Combustion products of composite materials for lighter weight vehicles and aircraft

Note: *= "Exposure conditions previously studied or currently proposed for study using automated neuropsychological testing".

ANAM was used to evaluate the effects of depleted uranium (DU) in Gulf War veterans with embedded DU shrapnel. Although DU is turning out to be a relatively safe heavy metal, uranium distributes to the central nervous system (CNS) and thus further investigation was warranted. The human studies have not revealed any clear effects [28], but this testing application raised issues of repeatability and learning effects (i.e. does the absence of improvement actually reflect a decrement in performance on some tests?), absence of a baseline testing comparison, and other confounding psychological stressors related to the initial traumatic wounding events.

6.4.4 Research Challenges

6.4.4.1 Lack of Objective Testing for Mood State

Mood disturbances including increased irritability and depression are prevalent reactions to many of these stressors including, for example, hypoxia [14], neuroglycopenia [13], and a variety of neurotoxicants [24], with resultant changes in motivation to perform well confounding the testing. At present, the subjective Profile of Mood States [29] is the primary instrument used in assessing mood status clinically; alternate tests that objectively quantify and distinguish characteristics such as curtness, lassitude, and helplessness from timed responses that also attempt to measure cognitive lapses in speed or accuracy have not yet been produced. Advances in methods to assess mood will be invaluable in neuropsychological evaluations.

6.4.4.2 More Data Generated than Can be Productively Analyzed

Researchers are drowning in the large volume of data produced by neuropsychological test batteries, with the large number of test options, variations in how the tests are administered, and more outcome variables such as speed and accuracy associated with each test. This seemingly infinite combination of outcome measures complicates interpretation of results, comparability with other studies, and study design, because of the increased likelihood of Type II error. If many of the available tests truly represent different functional axes, then bioinformatic methods being used to interpret genomics data may be needed. Alternatively, the testing could be reduced to five or six key tests with some kind of consensus standards. As an example, Kabat [4] found commonalities in some of the ANAM tests using principal components analyses, identifying three factors: processing speed/efficiency, retention/memory, and working memory. Bleiberg [30] produced similar factors in a separate analysis of data sets from sports concussion studies. Conceivably, one test with the strongest weighting against each factor could be selected as the starting point for an efficient test battery. The ANAM Research Evaluation System (ARES) battery devised to operate on a PDA is an example of a parsimonious starting point, consisting of simple reaction time, math processing, matching to sample, code substitution, memory search, and logical relations.

6.5 GULF WAR ILLNESSES – A MODEL OF POST-DEPLOYMENT NEUROPSYCHOLOGICAL CONCERNS

6.5.1 Post-Deployment Health Concerns

Cognitive status changes important to Soldier performance may also indicate early disease changes. As a result of the 1991 Persian Gulf War, neurological conditions and diseases have acquired specific importance in research on Soldier monitoring and protection. These range from chronic multi-symptom illnesses such as fibromyalgia, chronic fatigue syndrome, and multiple chemical sensitivities, to early indications of neurodegenerative diseases such as Parkinson’s Disease (PD) and amyotrophic lateral sclerosis (ALS) [31, 32]. Seemingly every conceivable etiology has been proposed for undiagnosed Gulf War illnesses, but the investigations have narrowed to just a few factors, including Soldier fitness and physical activity that may affect mental outlook and resilience [33], emotional stress influences [31], and specific neurotoxicants [25, 34, 35]. This thrusts neuropsychological testing into a central role in post-deployment health research.

6.5.2 Neuropsychological Complaints

Neuropsychological assessments of Gulf War veterans have been conducted in all of the major veteran study cohorts; unfortunately, only a few investigators have conducted rigorous testing, and most have relied on

symptom checklists. Every study has reported greater cognitive complaints for Gulf War veterans who deployed compared to non-deployed veterans, and nearly every study has described associated symptoms of fatigue and depression. For example, based on structured interviews, Iowa veterans who deployed to the Gulf had a higher prevalence of problems with memory and forgetfulness (>20% of respondents) compared to those who had not (6% – 8% of non-deployed respondents) [36]. There was a difference in the prevalence, but not in the nature of the complaints, with both deployed and non-deployed veterans reporting difficulty in concentrating and symptoms of depression. These and other studies indicate no unique syndrome or symptom complex, but a higher prevalence of illness in veterans who deployed to the Gulf, probably reflecting a general phenomenon affecting well-being of individuals after involvement in war or other significant events [37, 38].

6.5.3 Poor Correlation between Neurological Symptom Complaints and Neuropsychological Test Outcomes

Subjective complaints are not well reflected in neuropsychological testing performances. For example, Gulf War deployed veterans from Oregon and Washington states with undiagnosed illnesses revealed small differences in memory, attention, and response speed on a large battery of tests, but large differences in their psychological complaints compared to a sample of non-deployed veterans [39]. White et al. [40] studied two cohorts of Soldiers who deployed to the Gulf, and compared their responses to a group which was sent to Germany instead of to the Gulf. Detailed testing revealed no statistically significant difference in cognitive functioning, but mood complaints were significantly higher in Gulf War-deployed Soldiers. It was noted that Soldiers who reported that they believed they had been exposed to chemical or biological warfare agents demonstrated poorer performance on several cognitive tests, raising issues about motivation [40, 41]. Differences in cognitive test measures for Gulf War-deployed forces in the British studies disappeared after correction for multiple comparisons and adjustments for depressed mood [42]. Proctor studied the entire cohort of Danish forces deployed just after the end of hostilities in the Gulf and found no differences in detailed neuropsychological testing compared to non-deployed personnel, but did find a higher incidence of mood complaints (fatigue and confusion) [43]. The smallest but most highly studied and reported sample is that of Robert Haley and his colleagues who have focused their attention on 26 sick veterans compared to a similar number of healthy veterans. In Haley's sick veterans, intellectual and cognitive function were measurably decreased, but these findings may be confounded by major depressive illness, alcoholism, and post-traumatic stress disorder (PTSD) [44]. Another study that includes detailed neuropsychological testing and objective brain biochemical changes evaluated by magnetic resonance spectroscopy is currently underway with veterans from northern California. These data from various Gulf War cohorts raise an important issue of the influence of mood disturbances on cognitive function testing. It remains an open question about how well these domains can be evaluated in an automated push-button test in patients with fatigue and depression. Future ANAM developments will almost certainly have to consider testing other sensory systems.

6.5.4 New Studies in Force Health Protection

The 21-year Millennium Cohort Study initiated in 1999 and now comprising nearly 100,000 service members [45] has neurological health as the highest priority objective. However, there are no sensitive measures against which to base any associations, and the study must rely on diagnosed disease and subjective reports. The emergence of neurological symptoms and proposed pathophysiology involving psychological or toxicological causes opened the door to consideration of longer-term neurological diseases, especially those with a suspected environmental influence such as ALS and PD. Although data supporting any connection between military service exposures and these diseases is very thin because of the small number of cases and long disease latency, we are still ill equipped to obtain early detection of disease in these individuals. Neuropsychological testing is one important approach being employed in the investigation of Gulf War

illnesses and in studies on early detection and monitoring disease progression in PD in Army-sponsored research. It remains to be seen if neuropsychological testing can provide a sensitive indication of neurodegenerative disease changes ahead of functional imaging and physiological testing [46].

6.6 CURRENT AND FUTURE DEVELOPMENT OF THE ANAM TOOL

6.6.1 Exploratory Efforts

In May 2001, the Army sponsored a reunion of two dozen researchers who had piloted the development of neuropsychological tools under the sponsorship of the late Fred Hegge. Fred Hegge's vision was straightforward; we needed better tools to practically and usefully test brain function. During his tenure as the Director of the OMPAT, and later as the Director of the Military Operational Medicine Research Program, he was generous and insightful in bringing a range of talented scientists into the military neuropsychological testing family and providing enthusiastic mentoring and support to every new idea. This Pensacola reunion kicked off a new series of efforts to develop the ANAM and to coordinate the applications. It was especially important to impose some order on the chaotic mix of software versions and to standardize test procedures to enable comparisons of datasets between experiments. Every new application of ANAM seemed to include new and different test batteries and constant changes in test configurations. The Pensacola presentations recapitulated the lineage of ANAM back to the Walter Reed Performance Assessment Battery that had first automated commonly used neuropsychological tests [47], and concluded with current efforts to standardize the test procedures, software tools, and provide real-time analytic "back ends." The wide diversity of applications highlighted the broad importance of such a testing tool, with studies presented that ranged from Navy diving to radiation exposure by "liquidators" at Chernobyl; attempts to baseline Soldiers and Marines in psychology clinics; and specific applications to head injury, multiple sclerosis, and neurodegenerative diseases such as PD. Unfortunately, few of these studies have been published as full reports in the open literature, in part, because most of these have been opportunistic, with protocols that were not primarily guided by an ANAM-related hypothesis. The stressors and their effects on the individuals have not been well characterized, and the ANAM tests and methods have varied between tests and cannot be readily compared.

6.6.2 The Way Forward

The ANAM summit meeting led to a commitment by the Military Operational Medicine Research Program to champion the development of a family of standardized ANAM tools and their applications through military research studies, direct support to relevant extramural efforts, and leveraging of relevant grants and Congressional interest funding. This program support has spawned a range of research activities within the past few years. The development of the ANAM software has been headed by Kathy Winter (SPAWAR, Pensacola), supported through Army funding related to operational medicine and force health protection efforts. This effort was guided in part by CDR Dennis Reeves (Camp Pendleton Marine Base), and includes novel efforts by Dr. Tim Elsmore (Activity Research Services, San Diego) in the development of OS language palm version software, supported through a series of competitive Small Business Innovative Research awards. Independent verification and validation of testing procedures, including hardware and software analyses, has been provided for many years by Drs. Robert Schlegel and Kirby Gilliland at the University of Oklahoma, and this center has been licensed to continue ANAM standardization, distribution, and further development for the DoD. Specific applications in concussion studies by Dr. Joe Bleiberg (National Rehabilitation Hospital, Washington, D.C.), and in multiple sclerosis and other veterans disease issues by Dr. Robert Kane (Department of Veterans Affairs, Baltimore) have spearheaded the development of testing procedures and meaningful interpretation of results. Other diverse ANAM applications have ranged from development of drugs for treatment of incapacitating headache by Joe Bleiberg, to improvement of combat helmet designs in

parachute training and assessment of head injury and recovery following blast injuries by Dr. Deborah Warden (Defense Veterans Head Injury Program). Dr. Susan Proctor and MAJ James Ness formed a DoD-VA partnership to compare ANAM and other versions of neuropsychological tests in Soldiers stationed in Bosnia, pioneering the use of neuropsychological testing to monitor performance status in deployed forces. Most recently, Dr. Stephen Grate has been instrumental in introducing neuropsychological testing into several key Army grant awards for studies on co-morbidities in PD, notably important studies by Dr. Ken Marek (Institute of Neurodegenerative Disorders, New Haven, CT), and has shepherded the efforts to protect intellectual property rights of the various inventors so that the ANAM tools will remain freely available for government testing and applications.

6.7 CONCLUSION

The diverse military applications of ANAM testing described in this paper represent an ambitious agenda, but this does not imply that a single test battery or methodology is an appropriate fit to all these purposes. ANAM is intended to provide a family of standardized tests that can be translated across platforms, with batteries tailored to the appropriate use. Interpretation of test results will also vary according to the intended application, with very different standards and thresholds applied to epidemiological health screening tools and to individual fitness-for-duty tests. The first proof of the value of the ANAM investment will be any application where an ANAM test battery gains a reputation as a practical standard of testing, with proven predictive or diagnostic value. The most mature application of automated neuropsychological testing to-date has been in neurotoxicology assessments.

6.8 REFERENCES

[1] White, R.F. and Proctor, S.P. Research and clinical criteria for development of neurobehavioral test batteries. *J Occup Med* 1992; 34:140-8.

[2] Letz, R. Continuing challenges for computer-based neuropsychological tests. *Neurotoxicology* 2003; 24:479-89.

[3] Kane, R.L. and Kay, G.G. Computerized assessment in neuropsychology: A review of tests and test batteries. *Neuropsychol Rev* 1992; 3:1-117.

[4] Kabat, M.H., Kane, R.L., Jefferson, A.L. and DiPino, R.K. Construct validity of selected Automated Neuropsychological Assessment Metrics (ANAM) battery measures. *Clin Neuropsychol* 2001; 15:498-507.

[5] Hyams, K.C., Barrett, D.H. and Duque, D., et al. The Recruit Assessment Program: a program to collect comprehensive baseline health data from U.S. military personnel. *Mil Med* 2002; 167:44-7.

[6] Riley, D.L. Business models for cost effective use of health information technologies: lessons learned in the CHCS II project. *Stud Health Technol Inform* 2003; 92:157-65.

[7] Murnyak, G.R., Leggieri, M.J. and Roberts, W.C. The risk assessment process used in the Army's Health Hazard Assessment Program. *Acquisition Review Quarterly*, 2003; Spring: 200-216.

[8] Anger, W.K. Worksite behavioral research: results, sensitive methods, test batteries and the transition from laboratory data to human health. *Neurotoxicol* 1990; 11:627-717.

[9] Anger, W.K., Otto, D.A. and Letz, R. Symposium on computerized behavioral testing of humans in neurotoxicology research. *Neurotoxicol Teratol* 1996; 18:347-520.

[10] Wittels, P., Johannes, B., Enne, R., Kirsch, K. and Gunga, H.C. Voice monitoring to measure emotional load during short-term stress. *Eur J Appl Physiol* 2002; 87:278-282.

[11] Forster, E.M., Morrison, J.G., Hitchcock, E.M. and Scerbo, M.W. *Physiologic Instrumentation In The Naval Air Warfare Center Human-Use Centrifuge To Determine The Effects Of Cumulative +Gz On Cognitive Performance, Technical Report NAWCADWAR-956006-4.6*. Warminster, PA; Naval Air Warfare Center Aircraft Division, 1994.

[12] Hoyt, R.W., Reifman, J., Coster, T.S. and Buller, M.J. Combat medical infomatics: present and future. *Proc AMIA Symp* 2002; 335-9.

[13] Frier, B.M. Hypoglycaemia and cognitive function in diabetes. *Int J Clin Pract* (Suppl) 2001; 123:30-7.

[14] Fulco, C.S. and Cymerman, A. Human performance and acute hypoxia. In: Pandolf, K.B., Sawka, M.N., Gonzalez, R.R., eds., *Human Performance Physiology and Environmental Medicine at Terrestrial Extremes*, Indianapolis, IN: Benchmark Press, 1988; 467-495.

[15] Thomas, J.R., Ahlers, S.T., House, J.F. and Schrot, J. Repeated exposure to moderate cold impairs matching-to-sample performance. *Aviat Space Environ Med* 1989; 60:1063-7.

[16] Thomas, J.R., Shurtleff, D. and Schrot, J. *Administration of 1-tyrosine prevents cold induced memory deficits in Naval Special Warfare personnel, Technical Report.* Bethesda, MD: Naval Medical Research Institute, 1994.

[17] Ahlers, S.T., Thomas, J.R., Schrot, J. and Shurtleff, D. Tyrosine and glucose modulation of cognitive deficits resulting from cold stress. In Marriott BM, ed. *Food Components to Enhance Performance. An Evaluation of Potential Performance-Enhancing Food Components for Operational Rations.* Washington, D.C.: National Academy Press, 1994; 301-20.

[18] Sapolski, R.M. Why stress is bad for your brain. *Science* 1996; 273:749-50.

[19] Warden, D.L., Bleiberg, J., Cameron, K.L., Ecklund, J., Walter, J., Sparling, M.B., Reeves, D. and Arciero, R. Persistent prolongation of simple reaction time in sports concussion. *Neurology* 2001; 57:524-6.

[20] Bleiberg, J., Cernich, A.N., Cameron, K., Sun, W., Peck, K., Ecklund, P.J., Reeves, D.L., Uhorchak, J., Sparling, M.B. and Warden, D.L. Duration of cognitive impairment after sports concussion. *Neurosurgery* 2004; 54:1073-78.

[21] Penetar, D.M., McCann, U., Thorne, D., Schelling, A., Galinski, C., Sing, H., Thomas, M. and Belenky, G. Effects of caffeine on cognitive performance, mood, and alertness in sleep-deprived humans. In Marriott BM ed. *Food Components to Enhance Performance. An Evaluation of Potential Performance-Enhancing Food Components for Operational Rations.* Washington, D. C.: National Academy Press, 1994; 407-32.

[22] Balkin, T.J., Bliese, P.D., Belenky, G., Sing, H., Thorne, D.R., Thomas, M., Redmond, D.P., Russo, M. and Wesensten, N.J. Comparative utility of instruments for monitoring sleepiness-related performance decrements in the operational environment. *J Sleep Res* 2004; 13:219-27.

[23] Fiedler, N. Neuropsychological approaches for the detection and evaluation of toxic symptoms. *Environ Health Perspect* 1996; 104 (Suppl. 2):239-45.

[24] White, R.F. and Proctor, S.P. Solvents and neurotoxicity. *Lancet* 1997; 349:1239-43.

[25] Proctor, S.P. Chemical sensitivity and Gulf War veterans' illnesses. *Occup Med* 2000; 15:587-99.

[26] Tu, R.H., Mitchell, C.S., Kay, G.G. and Risby, T.H. Human exposure to the jet fuel, JP-8. *Aviat Space Environ Med* 2004; 75:49-59.

[27] McDaniel, K.L. and Moser, V.C. Utility of a neurobehavioral screening battery for differentiating effects of two pyrethroids, permethrin and cypermethrin. *Neurotoxicol Teratol* 1993; 15:71-83.

[28] McDiarmid, M.A., Engelhardt, S. and Oliver, M., et al. Health effects of depleted uranium on exposed Gulf War veterans: 10-year follow-up. *J Toxicol Environ Health A* 2004; 67:277-96.

[29] McNair, D.M., Lorr, M. and Droppleman, L.F. *Profile of Mood States*, San Diego, CA; Educational and Industrial Testing Service, 1971.

[30] Bleiberg, J., Kane, R.L., Reeves, D.L., Garmoe, W.S. and Halpern, E. Factor analysis of computerized and traditional tests used in mild brain injury research. *Clin Neuropsychol* 2000; 14:287-94.

[31] Clauw, D. The health consequences of the first Gulf War – the lessons are general (and for many patients) rather than specific to that war. *BMJ* 2003; 327:1357-8.

[32] Horner, R.D., Kamins, G., Feussner, J.R., Grambow, S.C., Hoff-Lindquist, J., Harati, Y., Mitsumoto, H., Pascuzzi, R., Spencer, P.S., Tim, R., Howard, D., Smith, T.C., Ryan, M.A., Coffman, C.J. and Kasarskis, E.J. Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. *Neurology* 2003; 61:742-9.

[33] Glass, J.M., Lyden, A.K., Petzke, F., Stein, P., Whalen, G., Ambrose, K., Chrousos, G. and Clauw, D.J. The effect of brief exercise cessation on pain, fatigue, and mood symptom development in healthy, fit individuals. *J Psychosom Res* 2004; 57:391-8.

[34] White, R.F. Service in the Gulf War and significant health problems: focus on the central nervous system. *J Psychopath Behav Assess* 2003; 25:77-83.

[35] Presidential Advisory Committee on Gulf War Veterans' Illnesses. *Final Report*, Washington, D.C.; U.S. Government Printing Office, 1996.

[36] Doebbling, B.N., Clarke, W.R., Watson, D., Torner, J.C., Woolson, R.F., Voelker, M.D., Barrett, D.H. and Schwartz, D.A. Is there a Persian Gulf War syndrome? Results from a large population-based survey of deployed veterans and nondeployed controls. *Am J Med* 2000; 108:695-704.

[37] Barohn, R.J. and Rowland, L.P. Neurology and Gulf War veterans. *Neurology* 2002; 59:1484-5.

[38] Hyams, K.C., Wignall, F.S. and Roswell, R. War syndromes and their evaluation: from the U.S. Civil War to the Persian Gulf War. *Ann Intern Med* 1996; 125:398-405.

[39] Storzbach, D., Campbell, K.A. and Binder, L.M., et al. Psychological differences between veterans with and without Gulf War unexplained symptoms. *Psychosom Med* 2000; 62:726-35.

[40] White, R.F., Proctor, S.P., Heeren, T., Wolfe, J., Krengel, M., Vasterling, J., Lindem, K., Heaton, K.J., Sutker, P. and Ozonoff, D.M. Neuropsychological function in Gulf War veterans: relationship to self-reported toxicant exposures. *Am J Ind Med* 2001; 40:42-54.

[41] Lindem, K., White, R.F., Heeren, T., Proctor, S.P., Krengel, M., Vasterling, J., Wolfe, J., Sutker, P.B., Kirkley, S. and Keane, T.M.. Neuropsychological performance in Gulf War era veterans: motivational factors and effort. *J Psychopath Behav Assess* 2003; 25:129-38.

[42] David, A.S., Farrin, L., Hull, L., Unwin, C., Wessely, S. and Wykes, T. Cognitive functioning and disturbances of mood in UK veterans of the Persian Gulf War: a comparative study. *Psychol Med* 2002; 32:1357-70.

[43] Proctor, S.P., White, R.F. and Heeren, T., et al. Neuropsychological functioning in Danish Gulf War veterans. *J Psychopathol Behav Assess* 2003; 25:85-93.

[44] Hom, J., Haley, R.W. and Kurt, T.L. Neuropsychological correlates of Gulf War syndrome. *Arch Clin Neuropsychol* 1997; 12:531-44.

[45] Ryan, M.A., Amoroso, P., Boyko, E.J., Gackstetter, G.D., Gary, G.C., Hooper, T.I. and Riddle, J.R. (2002). The Millennium Cohort Study. *Mil Med* 2002; 167(11):ii.

[46] Camicioli, R., Grossmann, S.J., Spencer, P.S., Hudnell, K. and Anger, W.K. (2001). Discriminating mild Parkinsonism: methods for epidemiological research. *Mov Disord* 2001; 16:33-40.

[47] Thorne, D.R., Genser, S.G., Sing, H.C. and Hegge, F.W. (1985). The Walter Reed performance assessment battery. *Neurobehavioral Toxicology and Teratology* 1985; 7:415-418.

Chapter 7 – HUMAN EXPOSURE BIOMARKERS: PERMETHRIN AS A MILITARILY-RELEVANT MODEL

by

Klaus-Gerhard Mross[1], Gina E. Adam[2], Daan Noort[3], and Jeannot Zimmer[4]

[1]Bundeswehr Regional Medical Command II, Occupational and Environmental Medicine, Diez, Germany
[2]U.S. Army Research Institute of Environmental Medicine, Natick, MA, U.S.A.
[3]TNO Defence, Security and Safety, Rijswijk, Netherlands
[4]Bundeswehr Institute of Medical Occupational and Environmental Safety, Berlin, Germany

7.1 ABSTRACT

Soldiers in operational environments are exposed to a variety of chemical substances. During deployment, they often move very rapidly from one "environment" to the other. Therefore ambient monitoring is very difficult and its outcome very complex to assess. Just as occupational and environmental assessments are becoming more common in industry, biomonitoring of chemical substances and their metabolites in body fluids of soldiers is the best way to calculate individual exposures. Urine is the preferred choice, because it is readily available, can be produced on a regular basis, and is simply processed for further analysis. For most of the exposures to militarily relevant substances, there exist laboratory methods to assess the soldiers' body burden. For distinct exposures, methods have to be developed, and additional scientific research is needed. These assays should be adopted in the military area, even when only the sample taking will occur in the deployment area and analysis and assessment will be done at home. Procedures for use in operational environments have to be introduced by national and international military institutions. International standards of laboratory and quality assurance procedures are required. The implementation of these procedures by international military organizations would have a great benefit for soldiers' health and military operability as well. Pre- or post-deployment medical examinations will only provide a significant benefit to soldiers' health if the implementation of overall and individualized biomonitoring is part of them. Biomonitoring procedures for short-term and long-term markers of permethrin exposures could act as a good model for other militarily relevant substances.

7.2 INTRODUCTION

Pre-, during, and post- deployment, soldiers suffer from a wide field of mechanical, physical, biological, chemical, and psychophysical stressors. From the perspective of occupational and environmental medicine, these burdens are difficult to assess, and the resultant adverse health effects often cannot be predicted or excluded. Additionally, possible unknown exposures complicate the situation. Recently, some military services have started conducting ambient monitoring during deployments. The interpretation of such ambient monitoring during deployments is difficult for several reasons: First, it is essential to have an idea about what substances will be present at the right time and the right place; and Second, deployed soldiers often move rapidly between unknown environments with additional danger of exposure. For that reason, additional measures have to be taken into consideration. As Kenneth Olden, Director of the *National Institute of Environmental Health* (NIEHS) stated: "Human exposure assessment is often the weakest link in risk assessment. … Adverse health outcome is a function of toxicity and exposure, both duration and intensity. … Environmental monitoring, which determines what's in air, soil, food, and water, is not equivalent to individual exposures." [1]. Thus, individual biomonitoring is essential, yet we must still determine what could

and should be measured, when, how, why, and what benefit this provides for deployed soldiers. Soldiers are often exposed to unknown chemicals. But more often they are exposed to well-known "home made" substances, such as fuels, explosives, lubricants, pesticides, etc. Biomonitoring can provide accurate information regarding exposure to chemical substances. Used properly, it may play an important role in military operational decision-making to benefit the soldier health.

7.3 HUMAN BIOMONITORING

Whereas ambient monitoring covers the amount and fate of chemical substances in the environment and in workplaces, biological monitoring is the determination of the levels of presumably toxic substances or their metabolites in body fluids or exhaled air. Determining their metabolites, adducts with proteins and DNA or other products of intermediate metabolism defines the contact and/or impact of these substances on the individual. Biological monitoring is a valuable way to protect an individual against the adverse health effects of chemicals in the workplace or in the environment. For that reason, it is necessary to implement a system of monitoring that allows assessments of exposures. These assessments may be either "ambient" or "biological" monitoring. Both should not be regarded as alternatives to each other. They should be considered complementary.

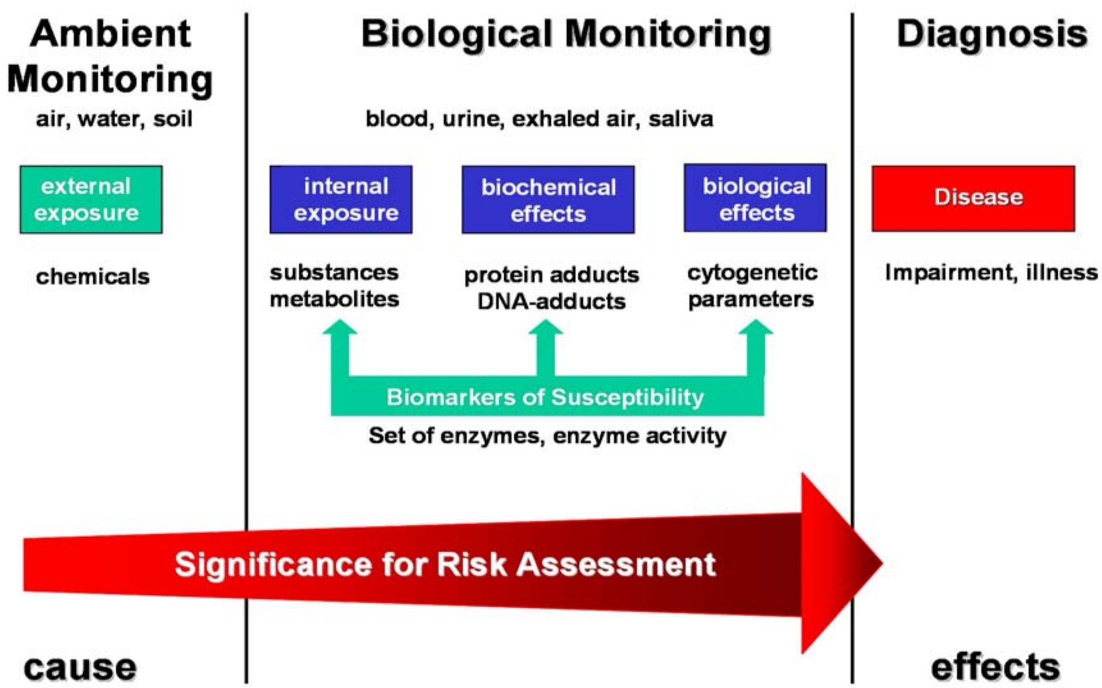

Figure 7-1: Monitoring Chemicals in Occupational and Environmental Sciences (modified from [2] by Mross).

Biological monitoring focuses on the measurement of interactions between chemical substances and the human "biological system". According to the *International Programme on Chemical Safety* (IPCS) three classes of biomarkers can be identified [3]:

- Biomarkers of Exposure — An exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism.

- Biomarkers of Effect — A measurable biochemical, physiological, behavioural or other alteration within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease (for an extensive overview see Chapter 2).

- Biomarkers of Susceptibility — An indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance (for an extensive overview see Chapter 8).

Biochemical effects, and to an even greater extent biological effects, are closer to the ultimate damaging principle and thus to the effects of the toxic substance than is dose monitoring. This means the predictive value of biological monitoring parameters increases from dose monitoring to effect monitoring. However, at the same time, the substance specificity decreases towards effect monitoring. With many effect parameters, such as chromosomal aberrations or DNA strand breaks, it can no longer be determined whether they occurred as a result of exposure to specific substances. In biomonitoring of exposure, this is quite different. Here the toxic substance can be determined specifically, but without providing any direct information about the effects [2, 3 – 5].

The goal of biological monitoring is to improve the prevention of diseases caused by toxic substances and their metabolites. Relationships must be established between parameters of biological monitoring and indicators of early stages of diseases.

7.3.1 Biomarkers of Exposure

7.3.1.1 Present Applications

Biomonitoring of exposure is used to confirm and assess the exposure of individuals or populations to a particular substance, providing a link between external exposures and internal dosimetry. After its introduction in the 1930’s, biological monitoring in occupational and environmental medicine increased steadily since the 1960’s, closely linked with the progress in analytical techniques and the growing knowledge of metabolic pathways [2]. Since that time, it has been an ever-growing important tool in medical health surveillance in the European Union and the United States. In Germany, since 1975 the DFG *Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area* has promoted the development of suitable, valid and tested analytical methods for biomarkers of exposure. Meanwhile, a collection of analytical methods has been produced that is like no other world-wide. In 1979, the DFG commission began to evaluate tolerable threshold limit values for the parameters of biological monitoring, such as *Biological Tolerance Values for Occupational Exposures* (BAT) and *Exposure Equivalents for Carcinogenic Substances* (EKA) [6]. In 1982, the *German Society of Occupational and Environmental Medicine* began to check the quality of the results of biological monitoring in intercomparison programs [7]. Today, an extensive intercomparison program for occupational and environmental toxicological routines is organized in Germany. In fact, more than 150 biomarkers of exposure (chemicals or their metabolites) can be detected by validated procedures in different body fluids or exhaled air [8]. Unlike in many other countries, biological monitoring is mandatory due to legal regulations on health and safety at work. For this reason there is an extended practical experience in the use of biological monitoring of exposure in occupational medicine. Biological monitoring

of exposure is applied to environmental toxicology as well, which is shown by epidemiological studies world-wide [9 – 12].

7.3.1.2 Main Prospects

In the view of the international IPCS working group: "biological markers of exposure pose unique advantages as tools for multimedia exposure assessment. They are highly sensitive indices of an individual's exposure to chemicals, since they provide a measure of the internal dose, account for all routes of exposure and integrate over a variety of sources of exposure. Therefore, they can represent past exposure (e.g. the presence of lead in shed teeth), recent exposure to an external source (e.g. volatile organic compounds in exhaled breath) and even future internal exposure sources (e.g. pesticides in adipose tissue). Furthermore, their use results in improved monitoring of total population exposure, characterization of individual and population exposures and evaluation of internal sources of exposure (see [12]). These markers are also useful surveillance tools for monitoring chemical exposure in both individuals and populations over time. Use of biological markers of exposure can improve the risk assessment process by providing a critical link between chemical exposure, internal dose and health impairment" [4].

The measurable parameters of biological monitoring are modulated by interindividual differences in detoxifying a substance: Enzyme polymorphisms, occurrence or absence of distinct enzymes or the metabolising capacities are the reason for differences in the performance of xenobiotic metabolizing enzymes. (see Chapter 8).

When absorption primarily occurs through skin, or when individual protective devices are used, biomarkers of exposure can provide reliable measurements of internal dose, which are useful to assess dose-response relationships.

The rational use of biomarkers of exposure needs sufficient toxicological knowledge about the substances toxicokinetics (absorption, absorption route, distribution, elimination, or may be accumulation), metabolism, their mode of action and adverse health effects. Different kinetic aspects, sometimes integrated in a physiologically based pharmacokinetic model (PBPK) help to define an appropriate biological monitoring strategy.

7.3.1.3 Integrated Access

Identification of suitable biomarkers associated with different toxic end-points or outcomes requires the collaboration of different scientific disciplines such as toxicology, occupational medicine, environmental medicine, molecular genetics, analytical and synthetic chemistry, and epidemiology. Only a small number of highly specialized scientists work in the area of exposure to toxic substances. Therefore international collaboration is necessary. Epidemiological studies should incorporate biological monitoring wherever possible. Epidemiology investigates relationships between exposures, biochemical, biological, and health effects. The description of the exposure situation according to the yes/no principle or with scores has proved to be unsuitable in many studies. Studies in which data for dose monitoring, effect monitoring, and health effects can be linked using epidemiological methods are urgently needed. With such a relationship, the advantages and disadvantages of the individual biological monitoring parameters can be exploited or compensated for. By identifying the parameters with the greatest diagnostic validity it should be possible not only to improve the prevention of adverse health effects, but at the same time to limit the efforts needed. By determining the dose taken up or the resulting biochemical and biological effects, epidemiological

investigations may provide more precise information about health effects. With the use of epidemiological methods in biological monitoring it should be possible to clarify pathomechanisms, to recognize the early stages of diseases, and also to include the genetically determined susceptibility in the estimation of the risk. However, if epidemiological methods are to be used in biological monitoring, the methodological shortcomings often observed (validity of the test, power of the study, selection of the control group, etc.) must be excluded [2, 5]. New sciences such as computational toxicology offer additional ways of supporting this integrated access.

7.3.1.4 Quality Assurance

Successful research in the field of biological monitoring needs analytical results comparable from laboratory to laboratory. This means that conditions must be created which lead to the minimization of possible influences on the analytical result. In the preanalytical phase all conditions for preanalytical procedures must be standardized and well documented. Main problems are the selection of appropriate biological media and sampling time. The analytical phase must be accompanied by an effective internal and external quality control. Important prerequisites are, for example, reference and standard substances and control materials. The availability of reference substances represents an ever-growing problem for biological monitoring. Chemists involved in the preparation of samples should therefore be included in the research in this area. Detailed descriptions of the complete analytical procedures, so-called standard operating procedures (SOPs), lead to the further reduction of possible influences on the analytical result [2 – 5, 8].

As far as the quality control of the analytical procedure in this field is concerned, the most progress has been made in dose monitoring (see above). In case of biochemical effect markers and biological effect markers, however, development is still at its beginning. Therefore, these activities must be included in appropriate research projects. The validity and reproducibility of all analytical procedures used in exposure biomonitoring must be checked according to the criteria of national or international standardization institutions, such as the German DFG commission [2 – 5, 8].

7.3.1.5 Future Needs

By determining toxicants and their metabolites in body fluids and exhaled air, the dose taken up can be evaluated specifically and sensitively. Due to the methods of instrumental analysis available today, it is possible to detect many substances down to the concentration ranges relevant to environmental exposures. The spectrum of parameters today includes different groups of chemical substances, such as metals, organic solvents, pesticides, aromatic amines and aromatic nitro compounds, and polyaromatic hydrocarbons. Nevertheless, this parameter spectrum must be continuously extended. In this context, the human metabolism of many organic substances, such as pesticides, must be clarified. The aim is to find those metabolites which are closest to the health risk. With the help of metabolic profiling, in addition to the ultimate toxic metabolite, interindividually different susceptibilities should be recognizable. Indispensable for both dose monitoring and the determination of protein and DNA adducts is progress in the sector of instrumental chemical analysis. ICP-MS should be included in the determination of metals. This method is suitable for the simultaneous detection of groups of refractory elements. The technological combination HPLC and multiple MS provides new measurement possibilities which are needed for the determination of adducts, specifically and sensitively, which have been enriched by prior handling with augmentation techniques such as PCR. This requires the use of tailor-made chromatographic separation phases such as *restricted access material* (RAM) or *molecular imprinting* (MIP) [2, 5].

7.3.2 Military Applications

Measurement of environmental exposures (i.e. ambient and biological monitoring) is essential for risk assessment and risk management. It is even more important for deployed military forces in uncharacterised (unknown) environments. The major goal of risk assessment is to prevent and predict disease from chemical or other environmental exposures. Until approximately 20 years ago, calculating an external exposure and assuming its correlation to the internal exposure determined the risk associated with environmental exposures. Today, there is an essential need to obtain pre-deployment and post-deployment biomonitoring data from military personnel. New strategies have to be developed in order to meet these new requirements, which should include the following points: knowledge base (Information sampling), process planning, coordination, preanalytics, transport, laboratory procedures, risk assessment, and reaction/countermeasures. Interoperability between the scientific specialities mentioned above, not only medical, is ultimately needed. It must be noticed that this process is time-critical. It should be well prepared and conducted under strict planning. During bi/multinational deployments, international scientific relations should be enhanced with bi/multinational access to the results. This saves time and resources and will bring much more benefit to the individual soldiers of all participating nations.

7.4 PERMETHRIN

7.4.1 Physical and Chemical Properties

Permethrin, chemically 3-Phenoxybenzyl(1RS;3RS)-3-2,2-dichlorvinyl-2,2dimethylcyclo-propancarboxylate, CAS-Nr C 52645-53-1, has a molecular weight of 391.3 g/mol. It is a clear light brown liquid with a weak characteristic odour. Melting point: 55.7-56.3°C (cis), 45.7-46.3°C (trans), solubility in water: 0.07mg/l (25°C), density: 1.0138 (25°C), vapour pressure: 2.15×10^{-8} mmHg (cis), 0.69×10^{-8} mmHg (trans). The industrial purity is approx. 90 – 99% [13].

7.4.2 Occurrence

Permethrin does not occur naturally. Like all pyrethroids, it is a synthetic analogue of the chrysanthemic acid (aliphatic ester compound from pyrethrum). The substance is used predominantly in agriculture. The quantity used in agriculture is approx. 10 – 20 g/ha (compared with several hundred g/ha in the case of other insecticides). For preventive medicine purposes, permethrin is used in different applications, such as fogging and spraying operations, impregnation of bed nets in malaria control operations, etc. It is for sale to outdoor enthusiasts at commercial outlets world wide under different brands. Its application has been favoured in almost all external and internal areas of vector control because of its distinctly lower toxicity to mammals than to insects. Permethrin is more effective against a broad range of pests than the more toxic organochloro, organophosphate, and carbamide insecticides. Because of its widespread use in agriculture, its occurrence in humans is ubiquitous. Biomonitoring studies have shown detectable permethrin metabolites in urine at very low concentrations within the general population in different countries [9 – 12, 14].

Environmental and toxicological qualities of permethrin are known from animal experiments and human exposures. They are collected and updated regularly in different freely available databases such as the *Hazard Substance Databank* (HSDB) and the *Integrated Risk Information System* (IRIS) [15, 16]. The substance is reviewed by international and national agencies, including the *U.S. Agency for Toxic Substances and Disease Registry* (ATSDR), the *International Programme on Chemical Safety* (IPCS), and the *World Health Organization* (WHO) [13, 17 – 19].

7.4.3 Military Use

There are a variety of applications for permethrin in the military arena. Several armies in the world use pesticide combinations to protect their troops from disease vectors such as mosquitoes, sand flies, fleas, ticks, mites, and lice. It is also important to shield the soldiers from the distraction and discomfort of biting bugs which impacts soldier morale. A complete review of usage and policies in military services has not been completed. A complete review on a standardized basis, to be updated permanently, is recommended.

The U.S. Army currently uses three methods for adding permethrin to uniforms. Two of these require the individual soldier to apply a solution to his uniform. The first of these is the *spray can procedure* developed in the 1980's which has a concentration of 0.5% and lasts for 5 or 6 launderings. This method involves a 6 oz. aerosol can which is used to spray a hanging uniform (i.e. not while on soldier's body, nor is the spray to be applied directly to skin) and then the uniform is allowed to dry. The second method is the *Individual Dynamic Absorption Application* (IDAA) kit, which includes plastic bags and two 9 ml bottles of permethrin in 40% concentration, each of which will treat one uniform piece. The procedure requires the user to combine ¾ canteen of water and one bottle of permethrin in one locking plastic bag, shake the bag to mix the chemical, add the uniform piece (BDU pants or top), close the bag and shake, and then leave to soak for 2 ½ hours or more; after which the soldier removes the garment from the bag and hangs to dry (product label). A third method is to be performed by trained personnel using a 2-gallon sprayer to apply 151 mL of permethrin at 40% concentration in two gallons of water for 50 seconds on each side to uniforms that are lying on the ground. This product is also applicable to bed netting. For both the IDAA kit and the 2-gallon sprayer application, the operator is required to wear protective gloves (the sprayer also requires a respirator: product label, MSDS). In both of these applications, after the uniform is hung to dry for three hours, no further application is necessary and permethrin protection is estimated to last 50 launderings, considered the life of the BDU. Additionally, instructions for all of the procedures state that uniform surfaces that touch the skin, such as underwear and headgear, are not to be treated. In addition to the methods outlined above which require the individual soldiers to treat their uniforms, the Army has developed an industrial application method. This method is the "preferred and recommended method" and is available through approved contractors in the commercial sector and provides for treated uniforms that the soldiers could buy or be issued. At this point, the factory impregnation with permethrin is available for woodland-pattern Battle Dress Uniforms (BDUs) and the desert camouflage uniform and has a permethrin treatment level of 0.125 mg per square centimeter. This treatment is also designed to last the life of the uniform, approximately 50 washings. To date, these uniforms are not yet available for distribution or purchase, subject to approval by the Army Uniform Board.

Current Army policy regarding the use of permethrin for deployed troops is elucidated in the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM) instruction letter for protection from biting insects. This guidance states that the best protection from biting insects (and thus, vector-borne diseases) is to use the DoD Insect Repellent System. This system is composed of three items: 1) a permethrin-treated uniform, 2) DEET on exposed skin surfaces, and 3) proper wear of the uniform (to include tucking in of undershirt and pants legs, no rolling of sleeves). The policy further instructs soldiers to sleep under permethrin-treated bed nets and to take all malaria prophylaxis pills as directed. These guidelines direct soldiers to use either the spray can or IDAA kit for permethrin treatment of their uniforms. Although the factory treatment procedure appears to be available, it does not seem to have been put into practice. In fact, an Armed Forces Pest Management Board (AFPMB) Information Paper states that no uniforms of any type have been factory-treated and distributed.

According to anecdotal reports from individuals who have been recently deployed to Iraq or Afghanistan, soldiers are being required at the CONUS Replacement Center (CRC) to pre-treat their uniforms with either

the IDAA kit or the spray can before leaving for the deployment theater. These individuals also reported that they did not see any evidence of permethrin treatment re-supply while they were in theater.

Safety analyses include: extensive animal studies; an Army-commissioned Committee on Toxicology report; an AFPMB Information Paper; a Health Hazard Assessments performed by/for the Army; and a risk/benefit analysis performed comparing risk of malaria/leishmaniasis to the toxicity of permethrin/chemical exposure. As for the safety and efficacy of permethrin treated uniforms, Army policy is that permethrin is effective in limiting biting insects and that there are no safety issues to soldiers wearing treated uniforms. Additionally, there are permethrin, deltamethrin, and cyfluthrin treatments available for impregnating bed nets or for spraying tents and curtains.

Current army policies, guidelines and safety analyses may be viewed and downloaded from the homepages of *U.S. Army Center for Health Promotion and Preventive Medicine* (CHPPM) or the *U.S. Armed Forces Pest Management Board* (AFPMB) [20, 21].

Faulde reported on the German Armed Forces approach which is similar to the US approach to include the recommendations that soldiers wear a permethrin treated uniform, use repellents such as diethyltoluamide (DEET) on exposed skin, and wear the uniform properly. A factory-based treatment method for the uniforms has been available since 2002. Faulde et al tested this permethrin polymer-coated uniform (Producer UTEXBel). It was found that uniforms impregnated in this way were effective throughout their useful life, ensuring protection of soldiers in the field from arthropod vectors, while showing less cross-contamination than those treated by the Insect/Arthropod repellent fabric treatment [22 – 24].

7.4.4 Toxicokinetics

Like all other pyrethroids, permethrin is lipophilic and has a very low vapour pressure. It is primarily absorbed by inhalation, generally by airborne dust; to a lesser extent through skin. Skin exposure may be predominant in pesticide operators. The general public is mostly exposed to permethrin residues in food and textiles [13, 17 – 19].

After absorption, permethrin is rapidly distributed throughout the body, mainly in the adipose tissue, stomach, intestine, liver, kidneys and the nervous system [17].

Once distributed, permethrin is rapidly and extensively detoxified by ester hydrolysis and hydroxylation in blood, liver, and other organs including significant amounts in the nervous system [12, 16]. The main metabolites are 3-phenoxy benzyl alcohol, which is further oxidized to 3-phenoxy benzoic acid (3-PBA), 3-(4'hydroxy)-phenoxybenzoic acid (4-OH)3-PBA and the two stereoisomers cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (cis-DCCA) and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (trans-DCCA) [13, 17].

After conjugation, the metabolites are excreted as glucuronides, sulphates or acetates primarily via the kidneys. A small amount is eliminated in the faeces [13]. The elimination time is biphasic, with a first phase within hours, whereas the second phase lasts for several days, indicating slower elimination from adipose tissues. Excretion normally ends within five days after terminating exposure, but can be delayed depending on the amount absorbed, the route of exposure and individual factors of susceptibility [17].

7.4.5 Health Effects

Permethrin has been used for many years. It is characterized by a moderate acute toxicity and does not show any evidence of long-term toxicity in humans [19]. Cases of systemic poisoning are rare and usually result

from accidental or intentional ingestion [17, 19]. No indication exists that permethrin has significant adverse effects on humans when used as recommended. It has induced skin sensations and paraesthesia in exposed workers, but these effects disappeared within 24 hours. Transient numbness, itching, tingling, and burning sensations have been reported in a small percentage of humans after dermal exposure to permethrin when it was used to treat head lice. Permethrin is not sensitizing to the skin, but may be slightly irritating to skin, eyes and nose [17].

Almost all ***systemic effects*** resulting from exposure to permethrin are related to the action of permethrin on the nervous system. It exerts its profound effect by prolonging the open phase of the sodium channel gates when a nerve cell is excited. Neurological signs typically result from acute toxicity. Low-level chronic exposure usually does not cause neurological signs in mammals, largely because of rapid metabolism and elimination. Data from animal studies do not indicate that permethrin significantly affects end points other than the nervous system, although changes in liver weight and metabolism sometimes have been used as an index of adverse effect levels. A few recent animal studies indicate the potential for adverse neurodevelopmental effects at dose levels at which other effects have not been reported. It should be considered in the interpretation of the neurotoxic effects observed in neonatal mice, consisting of changes in the density of muscarinic receptors at low doses: The unclear biological significance of the observed findings; the differences of mouse brain development process to that of humans, and the lack of standardization and comparability of the methods applied in neurotoxicological studies. Based on these considerations, further investigation of neurotoxicity is needed before conclusion can be drawn on this subject [19]. Data do not indicate that permethrin should be considered a carcinogenic concern to humans [17]. No data in humans are available regarding the potential for permethrin to cross the placental barrier and enter a developing fetus [17]. Permethrin was not mutagenic in the Ames test and was negative in two reverse mutation tests in *Escherichia coli* [13].

Studies of laboratory animals exposed to permethrin are summarized with *Non Observed Adverse Effect Levels* (NOAEL) and *Lowest Observed Adverse Effect Levels* (LOAEL) indicated [13, 15 – 19].

Standards and Guidelines for acceptable limits of exposure include:

World Health Organization (WHO) drinking water guideline	20 µg/L [13]
Food and Agriculture Organization/WHO accepted daily intake (ADI)	0.05 mg/kg BW/day [15]
Environmental Protection Agency Reference Dose (RfD)	0.05 mg/kg BW/day [16]

7.4.6 Permethrin Biomonitoring of Exposure

7.4.6.1 Short-Term Markers of Exposure

There are two main approaches to evaluating human exposure to permethrin. The first is by determining the levels of the unchanged substance in plasma or serum and the second is by determining its metabolites in urine. These two laboratory procedures reveal different information about permethrin exposure. Leng et al. report that urinary metabolites are the detoxified part of the pyrethroids and thus metabolite levels provide information regarding the magnitude of exposure. Conversely, plasma or serum determinations can indicate unchanged pesticide levels which could be related to symptoms. They further suggest that permethrin should only be determined in plasma for a few hours after a significant exposure. They have not found a correlation between metabolite concentrations in urine and symptoms after exposure in pest control operators. Some of their work is looking for a way to determine pyrethroid susceptibility. Their work seems to suggest that the half-life for an individual's metabolism (esterase activity) is related to symptoms and that half-life is increased if there is a co-exposure [25, 26].

The urinary metabolites serve as biomarkers of low and extremely low exposures and have been confirmed to be valid. Pyrethroids degrade to 3-phenoxybenzoic acid (3-PBA) and other metabolites, such as cis-3-(2,2-dichlorovinyl)- 2,2-dimethylcyclopropane-l-carboxylic acid and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane- l-carboxylic acid (cis-DCCA and trans-DCCA), cis- 3-(2, 2-dibromovinyl)-2,2-dimethylcyclopropanol- carboxylic acid (cis-Br_2CA), and 4-fluoro-3-phenoxybenzoic acid (F-PBA). Main permethrin metabolites are cis-DCCA, trans-DCCA, and 3-PBA, which are also metabolites for other pyrethroids. Therefore, Br_2CA and F-PBA is sometimes done to differentiate a permethrin exposure from that of deltamethrin and cyfluthrin. This assessment is easily conducted as all five metabolites may be determined in one laboratory run [27, 28]. Further it is possible to assess the exposure route (dermal, inhalation/oral) by calculating the ratio of DCCA and 3-PBA and the ratio of the DCCA stereoisomers [26, 29]. The development of analytical procedures for the measurement of the above-mentioned metabolites led to the method of Schettgen et al. using capillary gas chromatography with mass-selective detection (GC-MS) [27]. This method provides accurate detection of concentrations in urine for environmental exposures, but requires several hours to achieve acceptable detection levels. In military environments different levels of exposure exist, and it is difficult to detect exposures at the lowest levels. By introducing the LC/MSMS techniques into the routine, the detection level is increased but a broader range of concentrations can be determined within minutes. Also this procedure allows processing of a greater number of samples for screening purposes. Another benefit is the more preserving hydrolysis of samples by using glucuronidases than concentrated acids.

7.4.6.2 Longer-term Markers of Exposure

In contrast to the above methods, Noort et al. propose a different method for measuring exposure to pyrethroids. They suggest that since urine metabolites have a short half-life and thus clear the body quickly, a different method should be used to evaluate long-term or chronic exposure to pyrethroids. They posit that a further step in the metabolism of pyrethroids involves the formation of adducts with protein in plasma. They suggest that evaluation of albumin would provide a measure of *chronic* exposure to permethrin, something which cannot be evaluated via urine samples. It is well known that protein adducts of xenobiotics represent a much more persistent class of biomarkers than metabolites excreted into urine, having half-lives up to several weeks or months. For instance, protein adducts in human tissues have provided mechanistic insight into the epidemiological associations between smoking and cancer [30]. Accordingly, Noort et al have developed methods for biomonitoring of exposure to CW agents based on mass spectrometric analysis of such protein adducts, e.g. adducts of sulfur mustard with hemoglobin and albumin and of adducts of nerve agents with butyrylcholinesterase (for an overview see [31]). In view of its chemical structure, it should not be expected that permethrin itself will react with proteins to produce adducts. An ongoing study is focused on the (presumed) protein adducts of glucuronides of the two major carboxylic acid metabolites of permethrin, i.e. 3-PBA and cis/trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (DCCA; see Figure 7-2). Results have not yet been published [32]. Such adducts may serve as cumulative biomarkers for chronic exposure to permethrin. The O-acyl glucuronides represent a unique class of electrophilic metabolites, capable of reaction with nucleophilic sites in proteins. Numerous examples of these reactions have been documented in which the O-acyl glucuronides originated from drugs having a carboxylic acid moiety, such as several non-steroid anti-inflammatory drugs (NSAIDs), lipid lowering agents (gemfibrozil, clofibric acid), diuretic agents (furosemide) and the antiepileptic drug valproic acid [33]; (see [34] for an extensive overview). McKinnon and Dickinson [35] investigated the persistence of adducts of diflunisal- and probenecid-glucuronides with plasma proteins in volunteers. The adducts were still measurable at least one month after the parent drugs were undetectable. Several of the above-mentioned NSAIDS (e.g. benoxaprofen, zomepirac, ibufenac, tolmetin), have been withdrawn from the market because of severe adverse effects in patients, possibly due to antibody formation against the adducts with proteins, especially in the liver and kidneys [34]. Conjugation to glucuronic acid ("glucuronidation") by UDP-glucuronosyltransferase-mediated transfer of a glucuronyl moiety of UDP-

glucuronic acid to a nucleophilic site of a xenobiotic is one of the major Phase II detoxicification reactions. It renders the xenobiotic more polar which facilitates its excretion. This reaction takes place predominantly in the liver [36]. The most likely candidate for protein adduct formation by acyl glucuronides is human serum albumin (HSA), which is a rather abundant protein in the plasma (see e.g. [37, 38]). It has been demonstrated that the lysine 195 and 199 residues in the hydrophobic pocket of subdomain IIA of HSA are preferentially modified by various acyl glucuronides [39 – 41]. Adducts to lysine residues are probably rather stable in vivo. Interestingly, it was recently shown that these particular lysine residues are also highly reactive towards the acylating agent phosgene [35]. In this case an intramolecular adduct was formed, in which the lysine 195 and 199 residues were bridged intramolecularly by a urea-type chemical bond (with the carbonyl moiety derived from phosgene). Protein binding of glucuronides of benzoic acids that are structurally related to 3-PBA has been reported (see e.g. [42]). In more general terms, it appears that the degree of covalent binding to proteins of acidic drugs in man correlates well with the chemical reactivity of the glucuronides of these drugs [33]. It can be derived from the available data that adduct formation with proteins of glucuronide derivatives of carboxylic acid metabolites of permethrin is probable and will provide a useful biomarker to assess cumulative exposure to this pyrethroid. During preliminary experiments it was indeed found that the β-glucuronide derivatives of 3-PBA and DCCA are reactive towards several model peptides, including glutathione. In future work, it will be investigated whether similar adducts are formed in vivo, and whether they can be used for biomonitoring purposes (see also Chapter 10).

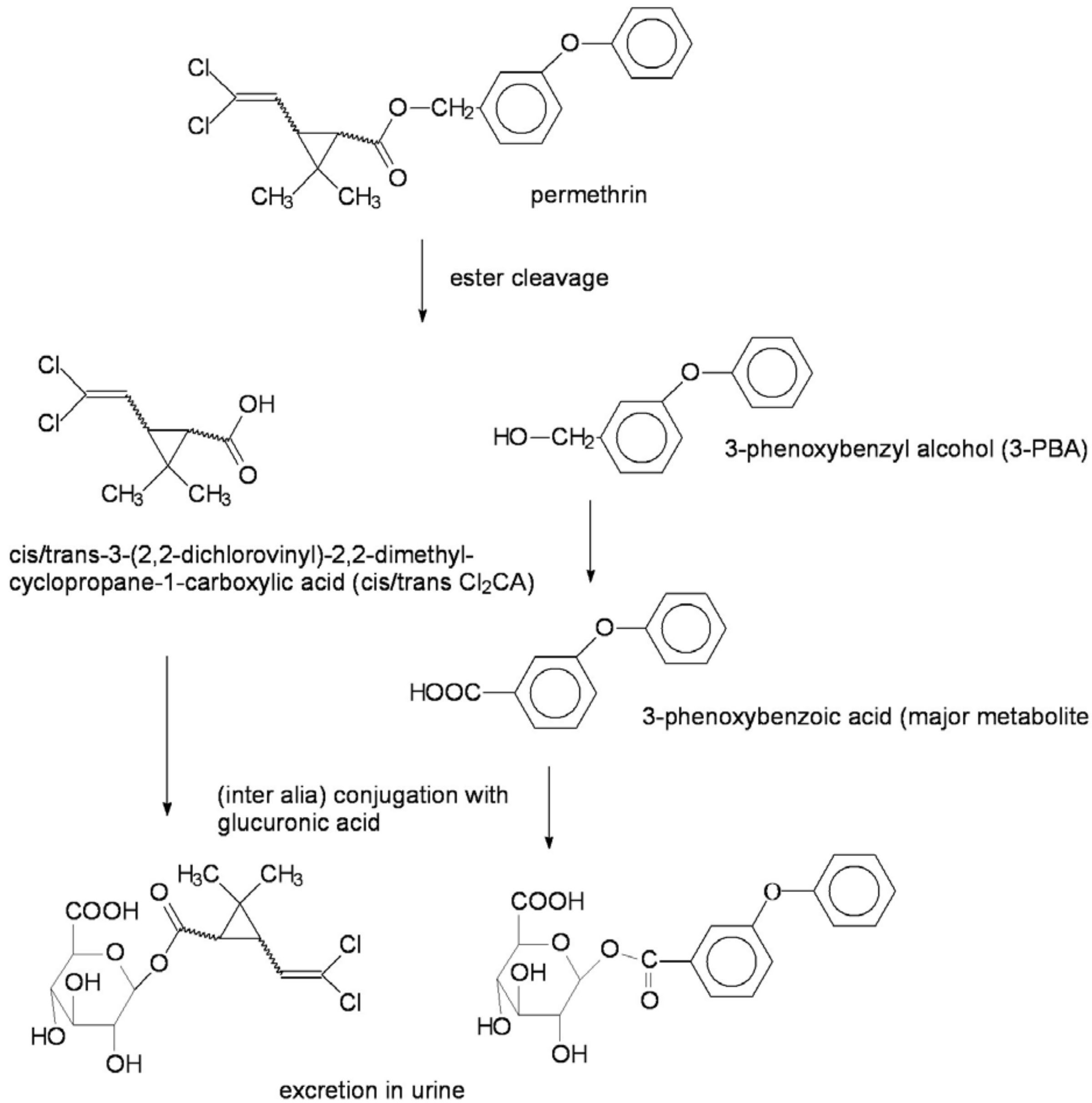

Figure 7-2: Metabolism of Permethrin (figure courtesy of Noort).

7.4.6.3 Bundeswehr Approach to Permethrin Biomonitoring of Exposure

Starting a risk assessment with a literature review focused on dermal permethrin absorption (for an extended overview on dermal absorption see [43]), several washing and cross contamination tests and intensive efficacy tests against different arthropods were conducted [22 – 24]. As a result the Bundeswehr decided to supply BDUs impregnated with the above mentioned UTEXBel procedure to troops deployed to areas with high vector risks. For the estimation of exposure Snodgrass predicted from dermal absorption studies in rabbits at a concentration of 0.125 mg/cm^2 in the fabric, a dermal permethrin transfer very far below the ADI of 0.05 mg/kg BW per day [44]. The *Subcommittee on Review of Permethrin Toxicity from Military Uniforms*

recommended conducting additional absorption and metabolism studies in small groups of soldiers [45]. Following this advice, the German Armed Forces Medical Service conducted a study of metabolite excretion in soldiers deployed to Afghanistan. The vector pressure in this country is so high that it was considered an ethical issue to exclude specific soldiers from wearing permethrin-impregnated uniforms. Therefore the "controls" were studied before the impregnated BDUs were supplied to the troops in Afghanistan. An exposed group, wearing the impregnated uniform for different periods of time, was tested later. Because of its very short half-life and very low exposures expected, permethrin was not measured in plasma or serum: permethrin metabolite levels in urine were measured instead. Spot urine was preferred, so that participation for the soldiers was simple. A specific medical questionnaire was answered; spot urine samples were taken, immediately frozen, and transported under stable temperature conditions from Afghanistan to Germany. The permethrin metabolites (3-PBA), cis-DCCA, and trans-DCCA were determined. To exclude confounding exposure to deltamethrin and cyfluthrin (often used for pest control operations during deployment), their specific metabolites F-PBA and Br_2CA were also determined. Laboratory procedures were conducted according to a procedure modified from Schettgen [27, 28]. On the basis of animal studies [17], dermal absorption studies following topical administration [29, 46], and the specific procedure of textile treatment by UTEXBel, a very low metabolite concentration had been expected. But the result was different. Whereas metabolite levels of the non-exposed soldiers were comparable to the non-exposed German general population, metabolite levels of the exposed soldiers exceeded these by far, but still well below the ADI (just one soldier with the highest metabolite concentration of all participants was close to 20% of the ADI). Therefore health impairments are rather unlikely.

Figure 7-3: Outdoor Permethrin Fogging Operation During Deployment (Potential Confounding Factor) (Photo courtesy of Zimmer).

Investigations showed that during deployment permethrin was used routinely in fogging and spraying operations, which could have confounded the results from the uniform exposure period. Therefore, another study was conducted in Germany under garrison conditions. To exclude confounders such as pest control operations, the participating soldiers came from two different garrisons [47]. Exposed soldiers and controls wore BDUs for four consecutive weeks. Spot urine samples were taken before the wearing period, after two and four weeks, and additionally four weeks after the wearing period. Exposed soldiers showed a concentration of permethrin metabolites in urine similar to those in Afghanistan. These results indicated an

exposure to permethrin when wearing the impregnated uniform. The mean trans-DCCA/cis-DCCA ratio in exposed soldiers was 2.7, [47, 48] which could be a sign of a more oral/inhalation exposure [25, 29]. Additionally, an elevated metabolite content was found in the urine of the exposed soldiers four weeks after exposure. The reason for this is not clear. Compared to the results of other human exposure studies [25, 29, 46] showing very short metabolite half-life and complete elimination within 150 hours after topical administration, the result of this study needs an explanation. The difference could depend on the exposure times (single/short term exposure vs. long time exposure), but further research is needed. Both studies indicate metabolite concentrations far above the reference levels of the general population in Germany, but well below the ADI. Permethrin-related health effects were not found [49]. Confounding deltamethrin and cyfluthrin exposures could be excluded in both studies. Both studies were conducted by the Institute of Occupational, Social and Environmental Medicine of the Johannes-Gutenberg-University, Mainz, Germany. Extensive results will be published soon in English. Preanalytics were prepared by the Bundeswehr Regional Medical Command II, Occupational and Environmental Medicine, Diez, Germany.

Taking into account the problem of vector-borne diseases during military deployments, the benefit of impregnating BDUs with permethrin is far higher than any potential risk of wearing this BDU.

There are different approaches for estimating pyrethroid exposures during military deployments depending on whether the exposure is more "occupational" or "environmental". The Bundeswehr is studying both types of pyrethroid exposure. Whereas the study described above had an "environmental" background; another study, currently being conducted by the *Bundeswehr Institute of Medical Occupational and Environmental Safety*, is monitoring other "occupational" pyrethroid exposures in addition to those usually expected during deployment.

Figure 7-4: Indoor Permethrin Fogging Operation During Deployment (Potential Confounding Factor) (Photo courtesy of Zimmer).

Deployment with a multi-national force brings an additional risk of pyrethroid exposure due to several factors. Although the desire to eliminate the vector-borne disease threat in the deployment area is often universal, the pesticides used and application strategies may vary greatly. Additionally, there is the possibility that application of these pesticides may not be coordinated which could result in soldier exposure to a number of chemicals in one treatment area. In those cases, estimating exposures can be misleading if this is not taken

into account. Under these conditions, biological monitoring of exposure requires a multilevel approach. Finally, some soldier populations may be further exposed due to their occupational risk, most notably pest control operators, preventive medicine specialists, and those working in NBC defense operations. To observe these soldier populations routinely by biological monitoring of exposure will possibly show additional "occupational" exposures. If elevated metabolite concentrations in urine are found, personal protective equipment must be improved. Therefore, qualified specialists in occupational and environmental health should participate in military deployments regularly to control the different paths of exposure and to find additional data by conducting questionnaires and taking samples from "occupationally" exposed soldiers. In such cases, it is recommended that representative data for long-term assessments be collected, especially when considering the repeated deployments (exposures) of many soldiers.

7.5 CONCLUSION

The healthy and well-trained soldier is the most valuable, yet most vulnerable, resource an army has. As much as is possible, he must be kept healthy before, during and post-deployment. Preventive measures including biomonitoring of exposure are mandatory. Scientific developments in biological monitoring have opened a new world for protection of soldiers. A wide range of chemical substances and their effects on humans is now measurable in the organism. Worldwide military deployments require that this new knowledge base is adopted, to optimize force health protection. To assess the risk of deployed soldiers, monitoring the environment and its influence on soldiers is necessary. Because ambient monitoring and biological monitoring each have their own weaknesses, applying them together in specific areas of exposure provides the best assessment approach of exposure. Strategies of exposure assessment have to be developed and implemented before deployment. Based on actual risk assessments, they should be adjusted before, during, and post deployment, dependent on the specific situation. Furthermore, a rational calculation of the balance between expenditure and benefit should be included in decisions to apply biomonitoring procedures.

Without basic knowledge and voluntary compliance of the soldiers and their leaders, every initiative of a monitoring strategy will be worthless. Although in several armies, under specific exposure conditions, some strategies have been successfully adopted from civilian occupational and environmental medicine, special procedures should be developed which cover the wide range of hazards and the specific living situations of deployed soldiers. Soldiers during deployment are not acting in industrial workplaces with their special standards and techniques. They are moving very rapidly from one adverse environment to another, facing unknown exposures, and are often not aware of imminent dangers. This is of major importance in mixed exposures, especially in situations where chemical, biological, physical, and psychophysical burdens occur in parallel. The scientific development of appropriate technologies and their adoption in military practice is very expensive. It should be managed cooperatively within the scientific military community of NATO to save time, money, and human resources. Civilian research benefiting military applications in the field of biomonitoring should be generously sponsored. For this development to occur, coordinated action and a steady flow of information are essential on national and international levels.

For the military, rapid assessment of exposures will provide soldiers and their military leaders with information critical for preventing or minimizing adverse effects of environmental contaminants, which might possibly degrade the troops' readiness significantly. Additionally, delayed effects, not readily apparent during a deployment, could cause decreased personnel readiness for future deployments as well as long-term health issues. The cost of these long-term health issues could be substantial. Therefore, the development of methods for the detection of toxic exposures of deployed military personnel is essential. Exposure biomarkers may become a key tool for assessing exposures of military personnel to environmental contaminants during deployments. Knowledge gaps should be defined, research should be conducted, and practical field

applications should be developed, as soon as possible. These field applications should be adjusted continuously to the state of the art of scientific research and the advances in laboratory sciences. Because of costs and benefits, international cooperation is needed. An international standardized toolbox should be introduced in the military area, which makes scientific knowledge easily applicable for use in military operations.

The experiences in biomonitoring procedures concerning permethrin exposures and their appropriate use during military deployments make it a good model for establishing practical tools in the detection of other militarily relevant substances.

At the current state of science, it is unlikely that there will be a single procedure which detects every possible exposure to our military forces. However, individual tests can detect specific exposures and effects. We must consider that unknown factors will continue to pose hazards to our deployed soldiers. Continuing to advance the science of biomonitoring will increase the ability to maintain our soldiers' health and safety in the face of environmental hazards and toxic harmful environments.

7.6 REFERENCES

[1] Martin, T.M. *Exposure Biomarkers in Military Deployments*. A research paper presented to the U.S. Army Command and General Staff College in partial fulfillment of the requirements for A462 Combat Health Support Seminar. Fort Leavenworth KS; U.S. Army Command and Staff College, March 2003. Online at: http://medicalservicecorps.amedd.army.mil/leader_development/cgsc_research_papers/martin-exposure_biomarkers.doc (Last accessed 5 June 2007).

[2] Angerer, J. Biological monitoring in occupational and environmental medicine – the present state of the art and future prospects. In: *DFG Biological Monitoring*. Prospects in Occupational and Environmental Medicine. Weinheim; Wiley-VCH, 2002; 5-15.

[3] International Programme on Chemical Safety (IPCS). *Biomarkers and Risk Assessment: Concepts and Principles*. Environmental Health Criteria 155. Geneva; World Health Organization, 1993. Online at: http://www.inchem.org/documents/ehc/ehc/ehc155.htm (Last accessed 5 June 2007).

[4] International Programme on Chemical Safety (IPCS). *Human Exposure Assessment. Environmental Health Criteria 214*. Geneva; World Health Organization, 2000. Online at: http://www.inchem.org/documents/ehc/ehc/ehc214.htm (Last accessed 5 June 2007).

[5] International Programme on Chemical Safety (IPCS). *Biomarkers in Risk Assessments: Validity and validation. Environmental Health Criteria 222*. Geneva; World Health Organization, 2001. Available online: http://www.inchem.org/documents/ehc/ehc/ehc222.htm (Last accessed 5 June 2007).

[6] Deutsche Forschungsgemeinschaft (DFG). *The MAK Collection for Occupational Health and Safety*. Weinheim; Wiley-VCH, 2006.

[7] The German External Quality Assessment Scheme (G-EQUAS). Available online at: http://www.g-equas.de/ (Last accessed 5 June 2007).

[8] Deutsche Forschungsgemeinschaft (DFG): Angerer J and Müller M. (Ends) *Analyses of Hazardous Substances in Biological Materials*. Vol. 1-9 (1992 – 2005). Weinheim; Wiley-VCH. Since 2006

(Vol. 10): DFG-Deutsche Forschungsgemeinschaft: The MAK-Collection for Occupational Health and Safety. Part IV: Biomonitoring Methods. Weinheim; Wiley-VCH.

[9] Berkowitz, G.S., Obel, J. and Deych, E., et al. Exposure to indoor pesticides during pregnancy in a multiethnic, urban cohort. *Environ Health Perspect* 2003; 111(1):79-84.

[10] Heudorf, U. and Angerer, J. Metabolites of Pyrethroid insecticides in urine specimens: Current exposure in an urban population in Germany. *Environ Health Perspect* 2001; 109:213-217.

[11] Heudorf, U., Angerer, J. and Drexler, H. Current internal exposure to pesticides in children and adolescents in Germany: urinary levels of metabolites of pyrethroid and organophosphorous insecticides. *Int Arch Occup Environ Health* 2004; 77(1):67-72.

[12] Centers for Diseases Control and Prevention (CDC). *Third National Report on Human Exposure to Environmental Chemicals*. Atlanta, Georgia; CDC, 2005. Available online at: http://www.cdc.gov/exposurereport/3rd/ (Last accessed 5 June 2007].

[13] Joint meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group (1999). *Toxicological Evaluations, Permethrin*. (Available online at: http://www.inchem.org/documents/jmpr/jmpmono/v99pr07.htm (Last accessed 5 June 2007).

[14] Bouvier, G., Seta, N., Vigouroux-Villard, A., Blanchard, O. and Momas, I. Insecticide urinary metabolites in nonoccupationally exposed populations. *J Toxicol Environ Health* 2005; B:485-512.

[15] Hazardous Substances Databank (HSDB). *Permethrin*. Available online at: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB (Last accessed 5 June 2007).

[16] Integrated Risk Information System (IRIS). Available online at: http://www.epa.gov/iris/ (Last accessed 5 June 2007).

[17] Agency for Toxic Substances and Disease Registry (ATSDR). *Toxicological Profile for Pyrethrins and Pyrethroids*. September 2003. Available online at: http://www.atsdr.cdc.gov/toxprofiles/phs155.html (Last accessed 5 June 2007).

[18] International Programme on Chemical Safety (IPCS). *Permethrin. Environmental Health Criteria* 94. Geneva; WHO, 1990, Available online at: http://www.inchem.org/documents/ehc/ehc/ehc94.htm (Last accessed 5 June 2007).

[19] World Health Organization (WHO). *Safety of Pyrethroids for Public Health Use and validation*. Environmental Health Criteria WHO/CDS/GCDPP/2005.10-WHO/PCS/RA/2005.1 Geneva; WHO, 2005. Available online at: http://whqlibdoc.who.int/hq/2005/WHO_CDS_WHOPES_GCDPP_2005.10.pdf (Last accessed 5 June 2007).

[20] U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM). http://chppm-www.apgea.army.mil/ (Last accessed 5 June 2007).

[21] U.S. Armed Forces Pest Management Board (AFPMB). http://www.afpmb.org/ (Last accessed 5 June 2007).

[22] Faulde, M.K., Uedelhoven, W.M. and Robbins, R.G. Contact toxicity and residual activity of different permethrin-based fabric impregnation methods for Aedes aegypti (Diptera: Culicidae), Ixodes ricinus (Acari: Ixodidae), and Lepisma saccharina (Thysanura: Lepismatidae). *J Med Entomol* 2003; 40(6):935-941.

[23] Faulde, M.K. and Uedelhoven, W. A new clothing impregnation method for personal protection against ticks and biting insects. *Int J Med Microbiol* 2006; 296 Suppl 40:225-229.

[24] Faulde, M.K., Uedelhoven, W.M., Malerius, M. and Robbins, R. Factory-based permethrin impregnation of uniforms: Residual activity against Aedes aegypti and Ixodes ricinus in battle dress uniforms worn under field conditions, and cross-contamination during laundering and storage process. *Mil Med* 2006; 171(6):472-477.

[25] Leng, G., Kuehn, K.H. and Idel, H. Biological monitoring of pyrethroids in blood and pyrethroid metabolites in urine: applications and limitations. *Sci Total Environ* 20; 1997(1-2):173-181.

[26] Leng, G., Leng, A., Kuehn, K.H., Lewalter, J. and Pauluhn, J. Human dose-excretion studies with the pyrethroid insecticide cyfluthrin: urinary metabolite profile following inhalation. *Xenobiotica* 1997; 27:1273-1283.

[27] Schettgen, T., Koch, H.M., Drexler, H. and Angerer, J. New gas chromatographic-mass spectrometric method for the determination of urinary pyrethroid metabolites in environmental medicine. *J Chromatogr B Analyt Technol Biomed Life Sci* 2002; 778:121-130.

[28] Angerer, J., Butte, W. and Hoppe, H.W., et al. Pyrethroid metabolites. In: Angerer J, Schaller, K.-H. (Eds) *Analyses of hazardous substances in biological materials, Vol 6, DFG*. Weinheim, GE:Wiley 1999; 231-254.

[29] Woollen, B.H., Marsh, J.R., Laird, W.J.D. and Lesser, J.E. The metabolism of cypermethrin in man: differences in urinary metabolite profiles following oral and dermal administration. *Xenobiotica* 1992; 22:983-991.

[30] Phillips, D.H. Smoking-related DNA and protein adducts in human tissues. *Carcinogenesis* 2002; 23:1979-2004.

[31] Noort, D., Benschop, H.P. and Black, R.M. Biomonitoring of exposure to chemical warfare agents: a review. *Toxicol Appl Pharmacol* 2002; 184:116-126.

[32] Benet, L.Z., Spahn-Langgut, H. and Iwakawa, S., et al. Predictability of the covalent binding of acidic drugs in man. *Life Sciences* 1993; 53:141-146.

[33] Bailey, M.J. and Dickinson, R.G. Acyl glucuronide reactivity in perspective: biological consequences. *Chem Biol Interact* 2003; 145,117-137.

[34] McKinnon, G.E. and Dickinson, R.G. Covalent binding of diflunisal and probenecid to plasma protein in humans: persistence of the adducts in the circulation. *Res Commun Chem Pathol Pharmacol* 1989; 66:339-354.

[35] Sallustio, B.C., Sabordo, L., Evans, A.M. and Nation, R.L. Hepatic disposition of electrophilic acyl glucuronide conjugates. Current *Drug Metabolism* 2000; 1:163-180.

[36] Presle, N., Lapicque, F., Fournel-Gigleux, S., Magdalou, J. and Netter, P. Stereoselective irreversible binding of ketoprofen glucuronides to albumin. *Drug Metab Dispos* 1996; 24:1050-1057.

[37] Qiu, Y., Burlingame, A.L. and Benet, L.Z. Mechanisms for covalent binding of benoxaprofen glucuronide to human serum albumin. Studies by tandem mass spectrometry. *Drug Metab Dispos* 1998; 26:246-256.

[38] Ding, A., Ojingwa, J.C., McDonagh, A.F., Burlingame, A.L. and Benet, L.Z. Evidence for covalent binding of acyl glucuronides to serum albumin via an imine mechanism as revealed by tandem mass spectrometry. *Proc Natl Acad Sci* 1993; 90:3797-3801.

[39] Ding, A., Zia-Amirhosseini, P., McDonagh, A.F., Burlingame, A.L. and Benet, L.Z. Reactivity of tolmetin glucuronide with human serum albumin. Identification of binding sites and mechanisms of reaction by tandem mass spectrometry. *Drug Metab Dispos* 1995; 23:369-376.

[40] Zia-Amirhosseini, P., Ding, A., Burlingame, A.L., McDonagh, A.F. and Benet, L.Z. Synthesis and mass spectrometric characterization of human serum albumins modified by covalent binding of two non-steroidal anti-inflammatory drugs: tolmetin and zomepirac. *Biochem J* 1995; 311:431-435.

[41] Noort, D., Hulst, A.G., Fidder, A., Van Gurp, R.A., De Jong, L.P.A. and Benschop, H.P. In vitro adduct formation of phosgene with albumin and hemoglobin in human blood. *Chem Res Toxicol* 2000; 13:719-726.

[42] Akira, K., Uchijima, T. and Hashimoto, T. Rapid internal acyl migration and protein binding of synthetic probenecid glucuronides. *Chem Res Toxicol* 2002; 15:765-772.

[43] International Programme on Chemical Safety (IPCS). *Dermal Absorption. Environmental Health Criteria 235.* Geneva; World Health Organization, 2006. Available online at: http://www.who.int/ipcs/publications/ehc/ehc235.pdf (Last accessed 5 June 2007).

[44] Snodgrass, H.L. Permethrin transfer from treated cloth to the skin surface: potential for exposure in humans. *J Toxicol Environ Health* 92; 1992; 35(2):91-105.

[45] Subcommittee to Review Permethrin Toxicity from Military Uniforms, Committee on Toxicology, Board on Environmental Studies and Toxicology, National Research Council. *Health Effects of Permethrin-Impregnated Army Battle-Dress Uniforms. NTIS/PB94-214400 NAP.* 1994. Available online at: http://www.nap.edu/books/NI000104/html/ (Last accessed 5 June 2007).

[46] Tomalik-Scharte, D., Lazar, A. and Meins, J., et al. Dermal absorption of permethrin following topical administration. *Eur J Clin Pharmacol* 2005; 61:399-404.

[47] Rossbach, B., Mross, K.G. and Scharnbacher, J., et al. Permethrin-impregnated uniform. A risk just for insects? *Proceedings of the 46. Annual Meeting of the German Society for Occupational and Environmental Medicine*. Hannover, March 25 – 28 2006, Voo7, 110-114 (on CD) [in German].

[48] Scharnbacher, J., Rossbach, B. and Mross, K.G., et al. Internal pyrethroid load in soldiers during deployment – influence of metabolite excretion. *Proceedings of the 46. Annual Meeting of the German Society for Occupational and Environmental Medicine*. Hannover, March 25 – 28 2006. P058, 553-555 (on CD) [in German].

[49] Scharnbacher, J., Rossbach, B. and Mross, K.G., et al. Stress reactions in soldiers wearing pyrethroid-impregnated vs conventional uniforms. *Proceedings of the 46. Annual Meeting of the German Society for Occupational and Environmental Medicine*. Hannover, March 25 – 28, 2006, P043, 507-509 (on CD) [in German].

Chapter 8 – BIOMARKERS OF INDIVIDUAL SUSCEPTIBILITY

by

Dr. Paul L. Knechtges[1] and Dr. Gudrun Cassel[2]

[1]U.S. Army Center for Environmental Health Research, Frederick, MD, USA

[2]Medical Countermeasures, NBC Defence, Swedish Defence Research Agency (FOI), Umea, Sweden

8.1 INTRODUCTION TO GENOTYPES AND BIOMARKERS OF SUSCEPTIBILITY

The biological basis for differences in individual susceptibility to diseases and responses to particular drugs has long been associated with biochemical variations of the same enzyme between individuals [1]. This phenomenon is known as polymorphic variation, and research over the past 60 years has directly linked the sequence and biochemical behavior of variant enzymes to the sequence of the genes encoding them. Such variations in the coding sequences of genes are the essence of individuality. Most of these variations are "neutral," occurring naturally in different populations without detriment to survival and good health. However, certain variations in individual genes or sets of genes can endow individuals with either susceptibility or resistance to disease or to other stressors.

The human genome consists of approximately 3 billion nucleotide sequences distributed among 23 pairs of chromosomes. Only a small percentage of the nucleotide sequences (~3%) encode the proteins essential for human life. The frequency of nucleotide polymorphisms is estimated to be about 1 in 1159 nucleotides in the regions of genes that encode proteins (known as exons). The total number of polymorphisms for enzymes important in the metabolism of drugs and chemicals is not known with certainty, but approximately 50 genes encode human cytochrome P450 enzymes [2]. In one of these genes that encodes an important cytochrome P450 (CYP2D6), over 90 polymorphisms have been discovered to date; some of these appear to be neutral, while others might be beneficial or detrimental in some circumstances [3].

The challenge for geneticists is to identify other relevant variations among the 3 billion nucleotide sequences of the human genome and link them to important phenotypic outcomes such as disease and drug metabolism. The combination of automated sequencing and clone-handling technologies with contemporary bioinformatics tools has made it possible to compare two or more individuals' genomes on a nucleotide by nucleotide basis. One can now find single nucleotide variations between two genomic sequences (single nucleotide polymorphisms or "SNPs"), although such efforts are very laborious. Considering there are approximately 10 million SNPs in the human genome, the task of linking particular SNPs to important phenotypic outcomes is daunting. Fortunately, groups of SNPs tend to be inherited together in blocks of chromosomal DNA called haplotypes. The number of haplotypes ranges from 300,000 to 600,000. By sampling only representative SNPs within a haplotype, the number of genetic markers is greatly reduced to a level that can be practically used in linkage studies to map, locate, and identify genes of importance. A human haplotype map, or HapMap, has been developed by an international consortium and is freely available to the public [4].

8.2 TECHNIQUES FOR DETERMINING GENOTYPES

Early methods of determining an individual's genotype ("genotyping") were based upon various known marker genes in specific regions of the chromosomes. They were laborious to perform and limited with respect to the

numbers of different genes that could be characterized and located. Over the past few years, great advances have produced simplified genotyping technologies using characterized collections of SNPs and haplotypes. A wide range of methods is currently available, and each year new methods are proposed. In general, current methods require extraction and amplification of the desired sequences of DNA followed by detection. The methods of detection include fluorescence, colorimetry, chemiluminescence, mass spectrometry, and others. Many genotyping methods are already commercially available. Table 8-1 lists examples of commercial suppliers and their products for SNP genotyping.

Table 8-1: Suppliers and Commercially-Available Products for SNP Genotyping

Kits	Microarrays
Applied Biosystems • SnaPshot Multiplex Kit • SNPlex Genotyping System • TaqMan SNP Genotyping Assays	Affymetrix • GeneChip HuSNP Probe Array • GeneChip Mapping 100K Array • GeneChip Mapping 10K Array • Par Allele TruTag Arrays
Beckman Coulter • MegaBACE SNuPe Genotyping Kit • SNPware Reagents Kits	GE Healthcare (Amersham Biosciences) • CodeLink SNP Bioarray System • CodeLink Human P450 SNP Bioarray and Reagent Kit
Invitrogen • SureScore SNP Genotyping System	Illumina • MHC Exon-Centric Panel • MHC Mapping Panel • Sentrix Human-1 Genotyping BeadChip
PerkinElmer • AcycloPrime-FP SNP Kit Promega • READIT SNP Genotyping System	Custom Arrays Printed by Core Labs
Pyrosequencing (Biotage) • Pyro Gold Reagents	**Other Methods**
Sequenom • MassEXTEND Mixes and/or Thermo Sequenase Enzyme Third Wave Molecular Diagnostics • Invader genotyping kits Other Suppliers and Products	SNP genotyping reagents developed in specific labs, i.e. “home-brew”

(Source: Bioinfomatics, LLC, 2005).

Additional technological advances are expected in the near future that will permit rapid, high throughput, and relatively inexpensive SNP genotyping of individuals. These technologies are essential for large-scale studies to establish associations between genetic biomarkers (haplotypes) and diseases or drug response [5]. Most current linkage research tends to be hypothesis-driven rather than discovery research. In the former approach, a set of SNPs (e.g. approximately 10) hypothesized to be important to the outcome variable is selected beforehand (*a priori)*, whereas discovery research uses many different SNPs (e.g. 100,000) and analyzes for associations with outcome variables afterwards (*posteriori*). Hypothesis-driven research can utilize technologies such as mass spectrometry, which can process thousands of samples (individuals) for less than 10 cents per sample. Discovery research is more expensive and requires more high throughput SNP/haplotype testing technology (e.g. microarrays) and greater computational power.

8.3 CLASSIC EXAMPLES OF GENOTYPING FOR OCCUPATIONAL HEALTH

8.3.1 N-Acetyltransferases (NATs)

N-Acetyltransferases (NATs) participate in the metabolism of aromatic and heterocyclic amines including important therapeutic agents. The aromatic and heterocyclic amines are frequently carcinogenic and are constituents of many environmental and occupational exposures. These amines are conjugated through acetylation by NATs and may become either detoxified or, conversely, bioactivated to become more potent carcinogens. It has been demonstrated that NATs have genetic polymorphisms producing enzymes ranging from slow to fast acetylators, which have also been associated with individual susceptibilities to various cancers [6]. The NAT polymorphisms may also predict the toxicity of certain drugs.

There are two prominent NAT isoenzymes (NAT1 and NAT2) encoded by different genes located adjacent to each other on human chromosome 8 [7]. Over the years, both the NAT1 and NAT2 genes have had many polymorphisms identified. Recently, Boukouvala, and Sim [8] conducted a complete structural analysis of the NAT1 and NAT2 genes and characterized the alternative mRNA transcripts. Prior to the capability to identify and characterize the NAT genes and transcripts, a number of probe drugs were used for phenotypic assessment of acetylation capacity in humans [6].

8.3.2 Glutathione S-Transferases (GSTs)

Glutathione S-transferases (GSTs) are important in the phase II metabolism of xenobiotic chemicals. These enzymes, combined with their substrate glutathione, have significant detoxification capacities. Four main classes of human GSTs have been identified: alpha (A), mu (M), pi (P), and theta (T); and each of these classes has one or more isoforms [9]. Polymorphisms in the cytosolic GSTs have been identified in humans and are likely to contribute to the interindividual differences in responses to xenobiotics [10]. Many research studies on the risks of cancer associated with different GST genotypes have serious shortcomings, because they only investigated the effects of homozygous null versions of the genes and did not examine individuals that were heterozygous for null and functional alleles. However, an increased risk of breast cancer was associated with heterozygosity in studies using novel methods, [11].

8.3.3 Glucose-6-Phosphate Dehydrogenase (G6PD)

Perhaps the best-known example of genetic susceptibility to an exposure is the association between erythrocytic G6PD deficiency and an increased risk of hemolytic anemia. G6PD regulates the distribution of glucose-6-phosphate between the glycolytic and shunt pathways to meet cellular needs for reductive biosynthesis and

cellular redox maintenance. Individuals who are deficient in G6PD activity are more likely to experience hemolytic anemia when exposed to fava beans, a number of drugs, and industrial or agricultural chemicals [12]. In the military, the G6PD activity of individuals is often assessed before administering the anti-malarial drug Chloroquine to avoid hemolytic anemia in G6PD-deficient individuals.

The G6PD gene is carried on the X chromosome, and the World Health Organization has classified the hundreds of possible variants into five groups (I – V) of activity in erythrocytes [13]. Progress in the molecular characterization of these variants have been expedited by polymerase chain reaction technology; many of the variants that were once considered distinct based upon biochemical properties have since been determined to be identical at the DNA sequence level [14]. The structural organization of the gene is now known, and recent research suggests that G6PD may play other subtle and important roles in biology, particularly with respect to antioxidant defense.

8.3.4 Paraoxonase (PON1)

Paraoxonase (PON1) is a member of a family of proteins which also include PON2 and PON3. They are serum proteins closely related to high density lipoprotein (HDL). PON1 not only hydrolyzes the active metabolites of some organophosphate pesticides such as chlorpyrifos and diazoxon but also the very toxic organophosphorous agents such as sarin, soman, and Vx [15 – 17]. Plasma PON1 activity in humans is genetically determined and has been found to vary 40-fold between individuals [18 – 20]. Furthermore, the gene frequencies for high or low metabolizers vary among groups with different ethnic and geographical origins [15]. To date, more than 160 polymorphisms have been described for these proteins. People with polymorphisms that debilitate PON1 activity and that limit AChE production are at greater risk for Parkinson's disease when exposed to agricultural insecticides [21].

8.4 THE FUTURE OF GENOTYPING IN MEDICINE

In the past, the greatest advances in medical genetics have been in identifying diseases that are associated with mutations in a single gene. Examples of these simple Mendelian diseases include Tay-Sachs disease and cystic fibrosis [22]. While discoveries of medically important variations within a single gene will continue, many heritable diseases clearly have more complex origins involving variations and interactions between multiple genes and environmental factors [23]. Deciphering these interactions to understand the genetic basis of disease will require special analytical tools and extensive research. Nonetheless, the journey to personalized medicine through genomics and genotyping is well underway.

In the near future, the impact of genotyping upon health care will likely be applications in diagnostics and drug development. Among patients who are prescribed a drug for a medical condition, a certain percentage will either not respond to the drug or will have an adverse reaction. If these patients can be identified by a genetic biomarker, then they can receive an alternative drug or treatment. Already, patients are receiving treatments for cancer on the basis of their genotypes, and more personalized treatments are anticipated. [24]. This type of personalized medicine is being spearheaded by the pharmaceutical industry, which can create new customized drugs and keep existing drugs on the market by identifying subpopulations for whom a drug is beneficial, detrimental, or ineffective.

Along with the HapMap – which will help identify and test for important genotypes – other technological advances will also make genotype screening more feasible. It is predicted that within a decade, the cost of sequencing the genome of an individual will cost $1,000 or less [25]. The implication of this achievement is that a patient's genome sequence will become part of the standard medical record for use in diagnosis,

treatment, and prognosis. For the military, the implications are even greater, because the medical records of military personnel tend to be better documented and computerized compared with civilian medical records, and military personnel are often prescribed mission-related prophylactic drugs and vaccines.

8.5 EPIGENETICS AND ENVIRONMENTAL EXPOSURES

The debate over "nature versus nurture" has existed throughout much of history and has been phrased in terms of science as "genes versus environment." However, recent evidence suggests that the relationship between genes and environment is not dichotomous but rather has a gene-environment interaction [26]. In a recent study conducted with 80 identical twins in Spain, it was determined that the differences in the regulation of gene expression between the twins became greater with the increasing differences in their environments (social and physical) [27]. Such differences in gene expression do not result from alterations in the DNA sequence, but from persistent transcriptional regulatory effects on gene expression. Environmental stimuli giving rise to such effects are called epigenetic factors and can result in phenotypic variation. Several mechanisms have been identified at the biomolecular level that account for epigenetic effects on gene expression, including DNA methylation and histone acetylation. These epigenetic modifications can be heritable through many cell generations [28].

The epigenetic phenomena complicate the application of genotyping in medicine and environmental health. Prior exposures and experiences could conceivably alter the onset and progression of environmentally-induced diseases. Epigenetic phenomena are still relatively poorly understood but may partly explain the differences in responses to environmental stress experienced by genetically similar individuals.

8.6 POTENTIAL GENOTYPING APPLICATIONS IN MILITARY POPULATIONS EXPOSED TO TOXIC CHEMICALS

Over the next two decades, the practice of genotyping will increasingly become part of mainstream medicine as more knowledge is acquired about clinically relevant polymorphisms and genotypes, and as patient care benefits are proven through research. Many of the civilian applications of genotyping will easily transition into military medicine. However, because military personnel face many environmental health hazards and receive medications and vaccines different from the civilian populace, genotype screening to protect troops from these unique exposures will present additional challenges for developing prevention strategies and doctrine on how to use this new knowledge and technology.

The military has the necessary infrastructure and accountability for its patient population to implement genotype screening on a large scale. But the military medical community has yet to develop policy or clinical practice guidelines for genotype screening. An exception to this generality is the use of G6PD screening of military personnel prior to administering anti-malarial prophylaxis. The revolution in pharmcogenomic medicine is coming, and it would be prudent for the military to proactively plan for it. Part of the planning process should include population-based and laboratory research to determine the underlying genetics of health and fitness traits that are relevant to military medicine.

Acknowledgement: We would like to thank Dr. David A. Jackson for his review and comments on this chapter to the report.

8.7 REFERENCES

[1] Garrod, A.E. The incidence of alkaptonuria: a study in chemical individuality. Lancet 1902; ii:1616-1620.

[2] Nelson, D.R. Cytochrome P450 and the Individuality of Species. Arch Biochem Biophys 1999; 369(1):1-10.

[3] Human cytochrome P450 CYP allele Nomenclature Committee. Available online at: http://www.imm.ki.se/CYPalleles/cyp2d6.htm (Last accessed 6 February 2007).

[4] International HapMap Project. Available online at: http://www.hapmap.org (Last accessed 6 February 2007).

[5] Shastry, B.S. SNPs and haplotypes: Genetic markers for disease and drug response (Review). Int J Mol Med 2003; 11: 379-382.

[6] Ambrosome, C.B. and Kadlubar, F.F. Biomarkers: Medical and Workplace Applications. Washington, DC: Joseph Henry Press, 1998; 189-210.

[7] Blum, M., Grant, D.M., McBride, W., Heim, M. and Meyer, U.A. Human N-acetyltransferase genes: Isolation, chromosomal localisation and functional expression. DNA Cell Biol 1990; 9:193-203.

[8] Boukouvala, S. and Sim, E. Structural analysis of the genes for human arylamine N-acetyltransferases and characterization of alternative transcripts. Basic Clin Pharmacol Toxicol 2005; 96(5):343-51.

[9] Ketterer, B. Glutathione S-transferase Polymorphism and Susceptibility to Cancer. In Ambrosome, C.B. and F.F. Kadlubar. Biomarkers: Medical and Workplace Applications. Washington, DC; Joseph Henry Press, 1998.

[10] Hayes, J.D., Flanagan, J.U. and Jowsey, I.R. Glutathione transferases. Annu Rev Pharmacol Toxicol 2005; 45:51-88.

[11] Roodi, N., Dupont, W.D., Moore, J.H. and Parl, F.F. Association of homozygous wild-type glutathione S-transferase M1 genotype with increased breast cancer risk. Cancer Res 2004; 64:1233-36.

[12] Jollow, D.J. and McMillan, D.C. Ethnic variation and genetic susceptibility: Glucose-6-Phosphate Dehydrogenase deficiency. In Ambrosome CB, Kadlubar FF, (Eds). Biomarkers: Medical and Workplace Applications. Washington, DC; Joseph Henry Press, 1998.

[13] WHO Working Group. Glucose-6-phosphate dehydrogenase deficiency. Bull World Health Organ 1989; 67:601-11.

[14] Ho, H., Heng, M. and Chiu, D.T. G6PD – An old bottle with new wine. Chang Gung Med J 2005; 28(9):606-612.

[15] Costa, L.G. and Furlog, C.E., (Eds). Paraoxonase (PON1) in health and disease: basic and clinical aspects. Norwell, MA; Kluwer Academic Publishers, 2002.

[16] Costa, L.G., Cole, T.B., Jarvik, G.P. and Furlog, C.E. Functional genomics of the paraoxonase (PON1) polymorphisms: effects on pesticides sensitivity, cardiovascular disease, and drug metabolism. Annu Rev Med 2003; 54:371-392.

[17] Shih, D.M., Gu, L., Xia, Y.R., Navab, M., Li, W.F., Hama, S., Castellani, L.W., Furlog, C.E., Casta, L.G., Fogelman, A.M. and Lusis, A.J. Mice lacking serum paraoxonase are susceptible to organophosphate toxicity and atherosclerosis. Nature 1998; 394:284-287.

[18] Mueller, R.F., Hornung, S., Furlog, C.E., Anderson, J., Giblett, E.R. and Motulsky, A.G. Plasma paraoxonase polymorphism: anew enzyme assay, population, family, biochemical and linkage studies. Am J Hum Genet 1983; 35:393-408.

[19] Davies, H., Richter, R.J., Kiefer, M., Broomfield, C., Sowalla, J. and Furlog, C.E. The human serum paraoxonase polymorphism is reversed with diazoxon, soman and sarin. Nature Genetics 1996; 14:334-336.

[20] Richter, R.J. and Furlog, C.E. Determination of paraoxonase (PON1) satus requires more than genotype. Pharmacogenetics 1999; 68:1428-1436.

[21] Benmoyal-Segal, L., Vander, T., Shifman, S., Bryk, B., Ebstein, R., Marcus, E.-L., Stessman, J., Darvasi, A., Herishanu, Y., Friedman, A. and Soreq, H. 2005. Acetylcholinesterase/paraoxonase interactions increase the risk of insecticide-induced Parkinsons disease. The FASEB J 2005; 19:452-454.

[22] Khoury, M.J., McCabe, L.L. and McCabe, E.R.B. Population screening in the age of genomic medicine. N Engl J Med 2003; 348(1):50-58.

[23] Collins, F.S., Green, E.D., Guttmacher, A.E. and Guyer, M.S. A vision for the future of genomics research. Nature 2003; 422:1-13.

[24] U.S. Food and Drug Administration. FDA clears genetic test that advances personalized medicine: Test helps determine safety of drug therapy. FDA News; 22 August 2005; 05,53.

[25] Guttmacher, A.E. and Collins, F.S. Realizing the promise of genomics in biomedical research. JAMA 2005; 294(11):1399-1402.

[26] Chakravarti, A. and Little, P. Nature, nurture and human disease. Nature 2003; 421(23):412-14.

[27] Fraga, M.F., Ballestar, E., Paz, M.F., Ropero, S., Setien, F., Ballestar, M.L., Heine-Suner, D., Cigudosa, J.C., Urioste, M., Benitez, J., Boix-Chornet, M., Sanchez-Aguilera, A., Ling, C., Carlsson, E., Poulsen, P., Vaag, A., Stephan, Z., Spector, T.D., Wu, Y.Z., Plass, C. and Esteller, M. Epigenetic differences arise during the lifetime of monozygotic twins. PNAS 2005; 102(30):10604-10609.

[28] Watson, R.E. and Goodman, J.I. Epigenetic and DNA methylation comes of age in toxicology. Toxicological Sciences 2002; 67:11-16.

Chapter 9 – NEUROTOXICOLOGICAL INTERACTIONS WITH PHYSICAL AND PSYCHOLOGICAL STRESSORS*

by

Karl E. Friedl[1], Stephen Grate[1], and Susan P. Proctor[2]
[1]U.S. Army Medical Research and Materiel Command, Fort Detrick, MD, U.S.A.
[2]U.S. Army Research Institute of Environmental Medicine, Natick, MA, U.S.A.

This chapter is based on a presentation "Lessons from the DoD Gulf War Illnesses Research Investment – Neuroepidemiology, Environmental Exposures, and Soldier Well-being" made at the NATO/PfP Workshop on Environmental and Industrial Health Hazards (EIHH) and Public Health Concerns in International Missions in Umea, Sweden, on 14 October 2004. A more comprehensive version is being prepared for publication.

9.1 ABSTRACT

Over the past decade, the U.S. DoD invested >$150M to investigate undiagnosed Gulf War Illnesses (GWI) and more than twice that amount in clinical management and related efforts with regard to the same issues. The research produced important new understanding of post-deployment health issues and potentially hazardous occupational, materiel, and environmental exposures. Gulf War Illnesses issues also created a new awareness of important neuropsychological and neurotoxicological interactions which were not new problems, but which represented a difficult and relatively untapped frontier in biomedical research in chronic multi-symptom illnesses. Some GWI topics such as blood-brain barrier integrity during stressful conditions and neurological effects of depleted uranium have been addressed, but others such as the neuroprotective benefits of aerobic exercise and psychosocial influences on individual stress resilience and resistance to neurotoxicity remain important areas of investigation. Current priorities for continuing investigation include:

1) Practical neuropsychological test methods;
2) Interactions between neurotoxic exposures and operational environments (e.g. exercise, heat, psychological stress);
3) Structure-function relationships of neurotoxins with neurodegenerative disease potential;
4) Objective correlates and biomarkers of neurological changes (e.g. neuroimaging with MRS); and
5) Markers of individual susceptibility.

9.2 INTRODUCTION

9.2.1 The Problem

The controversies concerning undiagnosed illnesses following the Persian Gulf War deployment (1990 – 1991) stimulated many useful discussions, much research, and improvements in force health protection that

* **Authors:** Friedl was the director of DoD GWI research program from 1994 to 2003; Grate has been the DoD technical expert for GWI and related neuropsychological and neurodegenerative disease research programs since 1997; Proctor is a key researcher in neuroepidemiology for the DoD, and previously conducted Gulf War Illness research at the Department of Veterans Affairs.

will ensure better medical care for the returnees from future deployments. Among the most important advances are thoughtful approaches to linking undiagnosed symptoms with new operational threats through biomonitoring of individuals, monitoring the environment, methods to increase resilience to operational stressors, and imminent breakthroughs in preventive neurology, which will be based on better understanding of interacting threats affecting neurological outcomes. For Gulf War veterans who are ill, the research has not provided treatment for their problems; after concerted research efforts by the DoD (since 1994), there remains no clear link between the common symptoms (neuropsychological changes, chronic fatigue, and arthralgia) [1] and presumptive exposures. The only difference between deployed and non-deployed troops in morbidity and mortality statistics was a 9% higher death rate, primarily attributable to motor vehicle accidents, and this excess mortality was dissipated after an additional seven year follow up [2, 3]. No new disease was discovered that was previously unknown to medical science, but there is a renewed emphasis on the still poorly understood family of disorders referred to as chronic multi-symptom illnesses [4, 5]. As a result of this research, other illnesses which may have occurred during of the deployment (e.g. Leishmaniasis), will be better understood. Safety evaluation of medical materiel has also been improved. It would be a surprising biological discovery if within a huge sample (~697,000 troops) there were zero adverse effects from the administration of a drug (e.g. pyridostigmine bromide), or vaccine (e.g. anthrax), or exposure to potent cholinesterase inhibitors such as DEET and permethrin. The question is, how do we identify at-risk individuals and how do we take safety to the next level, especially when casualties from enemy fire were extraordinarily low (372 deaths between Aug 1990-June 1991; 40% of these were combat casualties) and these wartime risks are actually lower than the potential risks associated with some of our own materiel.

Because no new syndrome was discovered, there is a perception that funding on Gulf War Illnesses research was money thrown into a black hole. In fact, many important findings and consequences have resulted from the spirited controversies produced by public discussion of poorly explained illnesses and from the resulting research. The DoD research to date has established new approaches to the early detection of changes in health status, new methods to monitor exposures, and advanced new understanding of how to assess the safety of medical prophylaxes and other materiel for use in operational environments (the interactions that go well beyond standard clinical testing for FDA approvals). Another important lesson is the appreciation of the impact of political and public pressures which can be supportive as well as hugely diverting when usually well-intended scientists enter the arena with extraordinary hypotheses to fill in the gap where research data is lacking. This huge gap in knowledge concerning neurological threats and outcomes is now better addressed by the DoD, and this should avoid a recurrence of the wide net of concerns that was thrown up in the absence of data on potentially harmful exposures and outcomes. These public concerns themselves produce adverse public health outcomes [6, 7]. Thus, the DoD's Gulf War Illnesses research program led to new discovery and emphasis on physical and psychological interactions affecting chronic multi-symptom illness; it addressed specific health risks and disease outcomes which had been previously overlooked; and it provided important lessons for the consequences of inadequate neurotoxicological data, biomonitoring of soldiers and environment, and health risk communication. These are all important themes of the HFM-057/RTG-009 research group.

9.2.2 Reviews of Gulf War Illnesses Research

The health issues to be investigated and the research that was accomplished has been extensively reviewed by national committees and panels including the National Institutes of Health [8], the Defense Science Board [9], the Centers for Diseases Control [10], Institute of Medicine's committee on Health Consequences of Service During the Persian Gulf War [11] the Presidential Advisory Committee on Gulf War Veterans Illnesses [12], the Office of the Special Assistant for Gulf War Illnesses, the Presidential Special Oversight Board for Department of Defense Investigations of Gulf War Chemical and Biological Incidents [13] and a series of Rand Reports [14]. Congress directed that the DoD and the Department of Veterans' Affairs provide $0.5M to

the Institute of Medicine annually between 1994 and 2003 to conduct epidemiological research on military and veteran populations. Many other investigator-led research reviews on Gulf War Illnesses have been published. In 1995, the first "working plan" for Gulf War research was published by the Persian Gulf Veterans Coordinating Board, composed of representatives from the DoD, VA, and HHS; this group coordinated federal efforts and published an annual report to Congress on Gulf War Illnesses research that continues today [15]. The DoD also funded several national conferences to facilitate and accelerate the exchange of emerging research findings in federally funded Gulf War Illnesses research in Washington DC, June 17 – 19, 1998; June 23 – 25, 1999; and January 24 – 26, 2001.

9.2.3 Phases of Gulf War Illnesses Research Management

The research on Gulf War Illnesses progressed in roughly three phases. President Clinton directed that "no stone should be left unturned" in the investigation of the poorly defined illnesses of returning veterans. In the absence of a clearly defined pathology and with a wide range of potentially neurotoxic exposures, many different testable hypotheses were examined. Table 9-1 highlights the range of studies that gained considerable public attention and demonstrates some of the proposed solutions to these problems, particularly a mystery condition where there was no clear case definition and no specifically identified health risk. Many of these hypotheses in the table were investigated in DoD-supported research. In the second phase of the research, the focus was narrowed to most probable threats and outcomes. Health concerns had been better defined in a three-tiered medical examination of sick veterans in the Comprehensive Clinical Evaluation Program (CCEP), with many conventional diagnoses eliminated as a single prevalent problem [16 – 18]. The research in this second phase addressed many issues which had not been previously addressed for specific known threats and diagnoses. This also included some large-scale interventions that tested immediate treatments of undiagnosed disease (long term antibiotic treatment, and cognitive behavioral therapy), as well as hypothesized mechanisms of illness which would be determined by the treatment outcomes. In the third phase, the effort focused on chronic multi-symptom illnesses, and possible mechanisms based on psychological and physiological interactions that impair performance and produce disease.

Table 9-1: Examples of Hypothesized Etiologies of Undiagnosed Symptoms of Gulf War Veterans

- Multiple chemical sensitivities (or, toxic induced loss of tolerance, TILT) (Claudia Miller)
- Bacterial infection (Edward Hyman)
- Mycoplasma infection (Garth and Nancy Nicolson)
- Sarin exposure resulting in progressive brain damage (Robert Haley)
- Neurotoxicity of DEET and interactions with other pesticides (Mohammed Abou-Donia)
- Squalene antibodies caused by squalene adjuvant in vaccines (Pam Asa)
- Inhalation toxicology threats from depleted uranium (Asaf Durakovic)
- Burning semen syndrome (Jonathan Bernstein)
- Sand-borne illnesses (A. Korenyi-Both)

This chapter briefly reviews the status and key findings of the research efforts that were managed by the Army on behalf of the U.S. DoD as part of the Gulf War Illnesses research program (1994 – 2003), and the efforts that have continued forward as part of other programs such as the Force Health Protection (FHP) research program and the Neurotoxin Exposure Treatment Research Program (NETRP). Key lessons learned that are relevant to this NATO panel are the problems that arise in the absence of comprehensive monitoring of baseline health and deployment exposures: efforts to develop useful biomarkers of exposure and effect after the fact produce an unsatisfactory confusion between validation of the markers and determination of actual health risks.

9.3 SPECIFIC ETIOLOGIES AND DISEASES INVESTIGATED

9.3.1 Diagnostic Criteria for Infectious Diseases – Leishmaniasis

Leishmaniasis was an early contender in the search for etiological agents of a Gulf War Illness because it was known to be an endemic problem in some of the areas where U.S. forces deployed and because of its reputation as the “great masquerader”, with elusive symptomatology mimicking other diseases [19]. After a relatively short period of excitement, including a brief ban on blood donations from all returning Gulf War veterans, the disease prevalence in returning soldiers was determined to be relatively low, with about two dozen cases of visceral Leishmania diagnosed [20]. Nevertheless, an entire program of studies was pursued with both extramural and intramural ($6M/4 years) efforts that culminated in a better understanding of the pathogenesis, vector control, diagnosis, and treatment of the disease. The program led to an advanced development effort to produce a commercially viable skin test which would be effective on both New and Old World Leishmania species and thus useful for future deployments in endemic areas of the world. Advances were made in the development of a serological test [21]. Funding to the basic and applied research program was terminated in 2002 to focus intramural research on higher priority endemic disease problems, although the problem of Leishmaniasis resurfaced with infections during more recent deployments to Afghanistan.

9.3.2 Other Infectious Disease Etiologies – Mycoplasma Infection

Other theories of infectious disease etiologies were kept in the public view through popular press stories, Congressional testimony, and internet sites. Mycoplasma infection was postulated as a central etiological agent by a well-known cancer researcher, Garth Nicolson. He and his wife developed their own unique PCR assay technique and published data suggesting an association between infection rate in sick Gulf War veterans and their family members and substantial resolution of symptoms after antibiotic treatment [22]. The DoD funded the training of other mycoplasma investigators at the Nicolson lab and supported a blinded study with multiple labs assaying the same samples from sick and healthy Gulf War veterans. The results and investigator interpretations were reviewed by a seven member external panel that recommended that no conclusions could be drawn from this study regarding mycoplasma and Gulf War veterans [23]. Another theory addressed bacterial infection [24], with very high dose intravenous antibiotic treatment for 36 patients at the Truoro Infirmary in Louisiana. The findings of this study have never been accepted for publication and no report has been made publicly available to detail adverse consequences and benefits from the high dose treatments. Infectious etiologies as a basis of Gulf War Illnesses were investigated in a randomized controlled trial with a full year of antibiotic treatment (200 mg/d, doxycycline). This multi-site VA and DoD study used 491 veterans who had deployed to the Gulf, had at least two out of three of the symptoms of Gulf War Illness (fatigue, pain, and cognitive complaints), and who tested positive for mycoplasma DNA. There was no difference in outcomes of treated and untreated sick Gulf War veterans [25]. An effort to assess possible infectious agents used another biomoniotring approach in a study of 118 military working dogs that had

deployed to the Persian Gulf. This study did not detect any new disease prevalence but did establish normal causes of death for two breeds, useful in future surveillance efforts [26].

9.3.3 Investigation of Neurotoxicity of Depleted Uranium

The depleted uranium (~60% less radioactive than naturally occurring uranium) used in some U.S. missile warheads, artillery and cannon rounds, and armor had never been assessed for health hazards to individuals on the receiving end of shrapnel and fragments. This became an immediate suspect in undiagnosed illnesses because of the perception that there might be a radiological risk, at least from fragments embedded in the tissues. Scientific questions were formulated around the neurological risks from heavy metal and threats to renal function based on a well-known rodent model of acute renal failure that uses uranyl nitrate. Congress also entered into the discussion and outlined studies for the DoD investigating all aspects of safety associated with depleted uranium. Thirty three veterans known to have embedded particles of uranium based on high urine uranium excretion have been closely monitored and have not revealed any significant health consequences attributable to the slow dissolution of the embedded particles [27]. Small changes in neuropsychological status of injured veterans with embedded DU disappeared in later testing [28]. Several significant animal studies were conducted to test the carcinogenicity and the neurotoxicity of DU. Soft tissue sarcomas were noted at the highest levels of exposure, with very large pieces of DU implanted in the muscle of rats [29]. Although this response was comparable to positive controls using radioactive Thorotrast and not observed with foreign body comparisons using tantalum, rats are known to be much more sensitive to radiation carcinogenesis than humans, and the significance of the very high exposure conditions to veterans remains in question. Neurotoxicological investigations indicate that uranium from embedded pellets does distribute to nervous tissue but no adverse effects have been detected [30]. No significant human health risks have been associated with depleted uranium exposure; however, this continues to be a subject of public discussion [31, 32].

9.3.4 Ruling Out Teratogenic and Reproductive Effects – DoD Birth Defects Registry

Adverse reproductive outcomes, including fertility problems in men and women, teratological outcomes in the offspring of exposed individuals, and pediatric cancers, can be sensitive indicators of toxicological exposures. The DoD lacked a birth defects registry database for use in investigations of patterns of adverse outcomes and to be able to rule out possible associations and to reassure soldiers if there were no associations. Several studies investigated birth defects in the children of Gulf War veterans, both for factors which could be transmitted through the male and in pregnancies of women who had deployed to the Gulf. A condition with a difficult and often incorrect diagnosis, Goldenhar syndrome, became a target of investigation. Although it was a statistical challenge due to low prevalence of the syndrome, eventually it was determined that there probably was not a significant association [33]. Other epidemiological studies concluded that there was no significant increase in birth defects or in any other specific defect [34, 35]. Two studies that focused on depleted uranium effects transmitted through the male were inconclusive. Fertility problems are much harder to assess on a large scale unless there is a major effect and these studies require detailed clinical workup of male and female partners. The lack of a practical biomarker measure of male infertility is particularly challenging in such studies, where variability in semen sampling and multiple factors involved in assessing potency make conclusions about reproductive toxicity very difficult (e.g. aerosolized lead exposure studies in field artillery operations). [36]. Self reported pregnancy outcomes from female veterans of the Gulf War were similar to non-deployed women although this type of self report and voluntary participation study is inherently biased in favor of differences [37]. Testicular cancer was also investigated as a disease that has been commonly ascribed to greater risks with military deployments and certain occupational toxic chemical exposures. One pilot study using cancer registries suggested an increased prevalence [38]. Other analyses indicated a

temporary increase, possibly related to deferment of care during deployment, with no difference in cumulative risks between deployed and non-deployed four years later [39]. Another condition, referred to as burning semen, briefly gained attention and was investigated but was rare and appeared to be no different than the frequency of this complaint in the civilian population as an immunological reaction that can occur in reproductive tracts of some individuals [40]. The Naval Health Research Center (San Diego, California) as a consequence of funded Gulf War research now maintains the DoD Birth and Infant Health Registry to monitor health of over 100,000 annual births to DoD personnel [41].

9.3.5 Searching for Adverse Effects of Pyridostigmine Bromide

Pyridostigmine bromide (PB) was distributed to US military forces in the Gulf War deployment to be used as a prophylaxis for the potential chemical warfare agent, soman. This was the first time that the drug had been used on such a large scale in healthy humans, although the actual useage rate was not well documented. As a cholinergic drug that had not been fully approved by the FDA for this application, this became an immediate suspect in the etiology of mystery neurological illnesses. Several important questions emerged that had not been specifically addressed in prior research including: PB penetration of the blood-brain barrier under high stress conditions; individual susceptibility to adverse effects of acetylcholinesterase inhibition; to include exposure of women (research had focused on effect in men) [42]; interactions with other compounds; interactions with other stressors such as physical stress [43]; and “bromism” from bromide accumulation. Extensive studies addressing each of these areas has substantially ruled out adverse effects of PB alone on the typical healthy individual but interactions with other compounds and stressors may be more complicated, as described in the next section. Rare exceptions in biological responses would not necessarily be detected, but have also been described [44]. Standardization of cholinesterase inhibition assays has been challenging and remains an important priority to produce reliable measures of status of an individual following drug administration or chemical exposure [45 – 47].

9.3.6 Cholinergic Interactions – DEET, Permethrin, and Pyridostigmine Bromide

Pesticides were deployed for approved uses in the Persian Gulf deployment but these were individually approved uses for safety and efficacy and did not consider safety with more complicated interactions. Less well examined before the deployment was the interaction of chemical prophylaxes, other materiel, and other physical and psychological stressors simultaneously affecting the nervous system. In some cases, there could be direct interactions of the chemicals and stressors (e.g. altered dermal or inhalation routes of entry during heat or exercise exposures). Studies by Hermona Soreq suggested that acute psychological stress (forced swim stress in rats) might alter blood brain barrier access to pyridostigmine [48, 49]. This led to a flurry of studies on physiological alterations produced by relevant stressors, looking empirically at interactions in various combinations and doses, as well as carefully designed studies that tested proposed mechanisms of interactions. The reported effect of stress on blood-brain barrier integrity has not been well supported but important differences in interactions of organophosphorus pesticides at the metabolic level are emerging [50, 51]. Soreq’s hypothesis was tested directly and no evidence has been produced to support the contention that there is a greater permeability in high stress conditions; however, effects can be enhanced by stress mechanisms through peripheral actions [52]. News headlines were made from reports that DEET might be more neurotoxic than previously recognized [53]. This claim depended in part on how brain histology was scored and relied on sensitive techniques that were not easily reproducible in the hands of other scientists. An important finding on the interaction of DEET and permethrin on dermal absorption [54] provided evidence for claims that toxicity of individual chemicals deemed below health hazard thresholds could act synergistically. Some empirical studies inferred interactions of neurotoxic chemical combinations (e.g. [55]). A study in rodents that attempted to recreate the psychological, physical, and neurotoxic chemical exposures

relevant to worst case exposures in the Gulf deployment found only a major effect from jet fuel exposures in rodents [56, 57]. A study that attempted to reproduce stressor (psychological stress and exercise) and chemical exposures (PB, DEET, permethrin) in humans in the laboratory found no effects in their outcome measures [58].

9.3.7 Jet Fuel and other Petroleum Products and Combustion Products of Tent Heaters

Petroleum products have been repeatedly investigated for occupational safety concerns but new studies were conducted in response to the Gulf War medical issues. Combustion products of unvented tent heaters were considered a possible source of illness and demonstrated some associations in self-reported exposures and pulmonary symptoms [59]. Some soldiers obtained their own tent heaters that were not intended for use in military tents without venting. A careful reproduction of a likely desert deployment scenario with actual vintage 1991 Persian Gulf deployed military tents and heaters, and using several conceivable fuels (kerosene, JP4), was established within a special clam shell enclosure to assess air flow, particulates and gases. The researchers concluded that, in the worst conditions, the first 20 minutes of warm up, the heaters produced high emissions of sulphates and air conditions equivalent to bad city air pollution [60]. Respiratory diseases were carefully assessed as part of the CCEP, especially because of the oil well fires that had been deliberately set during the war. No significant increase in pulmonary diseases or reduction in pulmonary function was detected. Careful assessments of the hazards by meteorologists and preventive medicine specialists, to include melding the data with a painstaking recreation of likely individual and unit exposures, as well as structured telephone interviews with a large sample of veterans, have not yielded any new information on health risks [61, 62]. A multistressor rat study that assessed combinations of chemicals and other relevant Gulf War stressors included JP4, the principal petroleum product used during the Gulf War. In all combinations, the groups receiving JP4, with or without other components, demonstrated significant neurotoxicological effects in a variety of tests in the Navy Toxicological Assessment Battery [56, 57]. Assessments of worst-case jet fuel exposure in certain job specialties in the Air Force, separate from the Gulf War Illnesses effort, have failed to produce any significant relationship to Gulf War Illnesses symptoms or any other important health effects [63]. Some neurological outcomes are of interest but have not been well addressed or fully analyzed in these previous studies. This has been discussed in the HFM-057/RTG-009 work sessions and new studies were developed to attempt to address JP8 exposures and potential neurotoxicologic effects.

9.3.8 New Inquiries into Health Consequences of Low Dose Chemical Threat Agents

Sarin was carefully studied in the program after revelations that a bunker with sarin had been destroyed by combat engineers, possibly generating a low dose airborne exposure to thousands of troops in the surrounding region. Although there were no acute symptoms, including miosis, noted in troops at the time, new inquiries into short term, very low dose (sub-miosis thresholds) exposure effects on long term health outcomes have been investigated. As with DU, no careful health hazards assessments had been conducted on chemical threat agents that were intended to deter or kill an enemy. Several controlled animal studies examined low dose sarin exposures and interactions with other chemicals or deployment stressors, although several of these projects encountered major challenges in establishing suitable laboratory procedures for studies with sarin. Low levels of sarin did not interfere with thermoregulation in rat inhalation studies [64]. In the same series of studies, repeated low level exposures produced delayed reductions in acetylcholinesterase and muscarinic receptors in the olfactory bulb and other parts of the brain; with the addition of a stressor (heat strain), the hippocampus was also affected [65]. Studies with marmosets demonstrated EEG changes (increased alpha frequency sleep-spindles) more than a year after a 5 hour low level sarin exposure, and less pronounced changes with pyridostigmine pre-treatment [66]. Studies of some of the Tokyo subway sarin attack victims indicate sleep disturbances and memory deficits ten years later, suggesting a consistency with the animal observations.

In an unusual role for the Institute of Medicine mandated by Congress, three large studies were conducted, one following up the long term health status of volunteers who had been deliberately exposed to chemical agents in studies at Aberdeen Proving Grounds in the 1970s [67], and another that examined morbidity and mortality of soldiers who had been in the vicinity of Khamisiyah during the demolition of the bunkers [68]; the third examined health care seeking behaviors of veterans [69]. The first study found no unusual pattern of illnesses in previously exposed research subjects; the second, the Khamisiyah study, reported an increased risk in brain cancer deaths among those considered exposed, based on exposure estimates and geographical locations at the time of the demolition of the Khamisiyah chemical munitions bunkers. One theory advanced by Robert Haley was that reduced paraoxonase enzyme levels in some individuals might make them more susceptible to sarin neurotoxicity, explaining lower paraoxonase blood levels and brain damage in symptomatic Gulf War veterans [70]; these findings await confirmation.

9.3.9 Observed Changes in Immunological Status and Vaccine Associations

Approximately 150,000 soldiers received FDA-approved anthrax vaccine: several studies found no relationship between immunizations and chronic multi-symptom illnesses. Further investigations of anthrax vaccine safety have been conducted to ensure safety after an expansion of the vaccination program in 1998 intended to protect the entire military [71]. Hotopf et al. reported that administration of multiple vaccines during deployment produced significant multisymptom illnesses not observed in other individuals receiving multiple vaccines. They concluded that there may be a psychological stress effect during deployment which affects the responses to vaccines [72]. This was further pursued with mechanistic investigations, testing the hypothesis that stress caused a shift towards increasing "Th2" T cell cytokine profiles including increased interleukin-10 secretion. The data suggested that sick Gulf War veterans were characterized by a "Th1" cytokine profile [73]. Another theory derived from experience with silicone breast implant court testimony was that the symptoms of Gulf War illnesses were the result of immunological dysregulation associated with squalene antibodies. Dr. Pam Asa hypothesized that squalene antibodies would be higher in sick Gulf War veterans and were a consequence of the use of squalene as an adjuvant in vaccine production [74]. While squalene has been used as an adjuvant in some foreign vaccines, no Gulf War veterans were known to have been administered vaccines produced with squalene adjuvant; nevertheless, this concept was popularized in lay press articles and became another etiology to investigate. A study by the Naval Health Research Center compared the prevalence of squalene antibodies in sick and healthy Gulf War veterans and results will soon be reported.

9.3.10 Neurodegenerative Disease Risks: Amylotropic Lateral Sclerosis (ALS)

Two different studies of Gulf War veterans each indicate a two-fold increased risk of ALS for servicemembers who deployed to the Persian Gulf. In one study, 40 cases were found for 696,118 deployed veterans (0.67/100,000), compared to 67 cases in 1,786,215 non-deployed veterans (0.27/100,000); the greater prevalence of ALS was highest in Air Force and Army (compared to Navy and Marine Corps) [75]. Another study reported a two-fold greater prevalence in Gulf War veterans by comparing 20 diagnosed cases in veterans to literature values for the general population [76]. A key problem with such studies of diseases with extremely low prevalence is the problem of case ascertainment and the effect of relative small differences between the compared groups. Any disease in the highly studied deployed Gulf War population is more likely to be identified ("ascertainment bias"). On the other hand, the relatively young age for disease presentation in these cases has been suggested to support a relationship to deployment exposures. On the strength of the first study by Horner, the Department of Veterans Affairs determined that it would provide service-related compensation to the small number of veterans who had deployed and developed ALS. In other reports, Dr. Haley suggested that other neurodegenerative diseases such as Parkinson's would also be expected in higher rates in deployed Gulf War veterans [77]. To date, no such associations have been detected. There are

strong associations between some neurotoxic chemical exposures and neurodegenerative diseases; however, currently available data are still relatively weak for any Persian Gulf deployment association and neurodegenerative disease. A retrospective study of older veterans compared military veterans to national rates, finding no differences overall in Parkinson's disease but a higher rate for ALS [78]. A recent review of existing data by a panel at the Institute of Medicine concluded that there is an association between the Gulf War deployment and increased risk of ALS.

Figure 9-1: Examples of Some of the Multiple Stressors Surrounding Soldiers During the Gulf War in 1990 – 1991. Warfighters are rarely subjected to one stressor at a time yet little of our research before the Gulf War had evaluated interactions relevant to military operational environments.

9.4 CHRONIC MULTI-SYMPTOM DISEASE AND WELLNESS

9.4.1 Case Definition of a Poorly Defined Neurological Outcome

The first complex of symptom complaints that lacked a standard diagnosis emerged from a survey of an Army reserve unit by a young preventive medicine officer, Bob DeFraites [79], although they did not appear in standard diagnostic codes [80]. Other studies quickly expanded the effort, including surveys focused on regional locations/specific states [81, 82] and military units such as the Seabees [83]. An Air Force survey by a CDC researcher, Fukuda, established a case definition of the most common undiagnosed symptom complexes which specified two out of three complaints of neurocognitive deficits, fatigue, and arthralgia [1]. The Fukuda definition is the one that is now most commonly used to classify otherwise undiagnosed but sick Gulf War veterans as suffering from "Gulf War Illness," a category of poorly defined chronic multisymptom

illnesses that include recognized diseases such as chronic fatigue syndrome and fibromyalgia. Eventually, the DoD-funded effort included several longitudinal cohort studies, notably, a Department of Veterans Affairs national survey [84], continued analysis of the DoD Gulf War registry population [62] a military sample centered at Fort Devens, Massachusetts [59], a state-wide military sample in Iowa [85], and a study of British forces [86]. The most important findings from these studies were that Gulf War era veterans who had actually deployed to the Gulf registered more complaints on all symptom checklists than individuals who did not deploy or other era comparison groups.

9.4.2 The Haley Hypothesis

Robert Haley published detailed studies of sick veterans based primarily on a set of symptom complexes he derived from one of six clusters in an epidemiological survey of sick veterans: "Haley Syndrome 2" (including detailed clinical studies of approximately 26 sick Gulf War veterans compared to a similar number of healthy comparison veterans who had deployed to the Gulf) [87, 88]. His conclusion was that these illnesses represented a neurodegenerative disease involving damage to the right basal ganglia that he attributed to neurotoxic chemical exposures unique to the Gulf War deployment. This became a very public discussion when Ross Perot championed the concept and suggested that the DoD was overly focused on psychological stress rather than toxic chemical etiologies. A special independent panel on Gulf War Illnesses (the Presidential Special Oversight Board on Gulf War Illnesses) appointed by President Clinton reviewed the Haley data and concluded that the findings needed to be confirmed by other researchers. A large DoD-funded study, which has not yet been published (and which involved Dr. Haley as a consultant on the methodology) studied a new and larger sample of Gulf War veterans and included careful assessment of potentially confounding problems such as alcoholism, severe depression, and PTSD. Another federal advisory panel, the VA Research Advisory Committee, was established by order of Congress to advise the Department of Veterans Affairs on further research in Gulf War Illnesses. Their most recent recommendation to the Department of Veterans' Affairs is a research solicitation for \$15M/year to continue research led by Dr. Haley on topics primarily related to neurotoxic chemical etiologies and with specific exclusion of studies focused on psychological factors [89,90]. The publicity surrounding the Haley data directly contributed to a renewed effort by the DoD to investigate Gulf War medical issues through the establishment of a four year \$20 million/year research effort on Gulf War Illnesses. Much of the research discussed in this chapter was funded as a result of this program. Unlike the focus of the VA RAC to prove a simple "smoking gun" etiological agent such as low-level sarin exposure, the DoD effort has diverged to multifactorial studies of chronic multisymptom illnesses, including both psychosocial factors and neurotoxic chemical exposures.

9.4.3 A Focus on Chronic Multisymptom Illness

Several researchers have exhausted their Gulf War study cohorts, finding that many veterans even after entering studies of illness claim to be better or recovered, but many Gulf War veterans still report symptoms of illness and these illnesses have lasted longer than those in veterans from other deployments [91]. The new emphasis on chronic multisymptom illnesses has led to some new understanding in this area, regarding the importance of activity and continued engagement following exposure to major life events such as a wartime deployment. Other Congressional special interest programs specifically supporting efforts in fibromyalgia and chronic fatigue syndrome have been linked to the Gulf War Illnesses focus in this area [92]. A treatment center at Walter Reed Army Medical Center has developed outcomes from the research to develop cognitive behavioural therapy approaches for symptomatic soldiers. A large study by VA and DoD on cognitive behavioural therapy of undiagnosed Gulf War veterans produced some apparent benefits [93]. Other researchers have focused on sympathetic changes such as orthostatic hypotension [94], and other neurological indicators [95]. Specific odors and involvement of the olfactory system triggering biological responses (e.g. cadaverine in

relation to exposure to psychologically traumatic events such as mass gravesites), and a wider variety of odors such as those that may trigger trigeminal responses through irritant and other chemesthetic pathways that may condition future neuroplastic responses, were also investigated [96 – 98].

While no new disease that was previously unknown to medical science has been discovered through the Gulf War Illnesses research effort, the many scientific discoveries and accomplishments (Table 9-2) have advanced military medical science and improved the ability to respond to future deployment health issues.

Table 9-2: Some Key Research Accomplishments of the DoD Gulf War Illnesses Research Program

• Ruled out numerous etiologies as likely causes of significant unexplained illnesses
• Developed Leishmania diagnostic skin test
• Determined that depleted uranium is less of a health hazard than initial concerns suggested
• Investigated stress effects on blood brain barrier integrity
• Refined safety assessments of pyridostigmine bromide for chemical agent prophylaxis
• Improved neurotoxicological testing methods
• Advanced knowledge of immune responses to vaccines in stressful environments
• Refined baseline testing such as predeployment health screens and neuropsychological testing
• Investigated safety of combinations of pesticides, jet fuel, pyridostigmine bromide, low level sarin, and other Gulf War deployment stressors, developing new understanding of important interactions
• Established new epidemiological cohorts and registries for more responsive investigation of future deployment and occupational health concerns
• Advanced the field of chronic multisymptom illnesses pathophysiology, diagnosis and treatment

9.5 NEUROTOXIN EXPOSURE TREATMENT RESEARCH

A related Congressionally-sponsored effort, the Neurotoxin Exposure Treatment Research Program (NETRP), began with $25M in 1997. This was intended as a Parkinson's research program but the investigation of Parkinson's Disease as both a neurological disease model and with pathogenesis directly relevant to military health threats is a very important extension of the efforts to correct and prevent issues identified in the Gulf War Illnesses from being experienced in future deployments. A study demonstrating low genetic concordance for Parkinson's Disease based on twins (compared to some other neurodegenerative diseases such as Alzheimer's that have a much stronger genetic basis), supported the notion that this could be an important model for the DoD to focus neuroscience research on, with dual use (military threats and civilian disease treatment) applications. Head injury, neurotoxicological threats including pesticides or nerve agents, chronic psychological stress, and traumatic stress are all relevant military stressors with neurological health and performance implications of great importance to the DoD, having common pathogenic mechanisms such as oxidative stress [99]. Many of the important projects underway today have direct military relevance and relevance to the efforts of this NATO panel including identification of biomarkers of early neurological changes. Projects concerning specific neurotoxic threats (pesticides including permethrin, PCBs, and methyl mercury) and fundamental studies on neural plasticity including mechanisms to accommodate changes in cholinergic function provide insight for development of enhanced protection of soldiers. An improved

understanding of the pathogenesis of neurodegenerative diseases will allow the DoD to better protect soldiers by physiological monitoring and to specifically steer away from inadvertently harmful exposures. Neuroepidemiology provides an opportunity to test this understanding as it develops and to point the way for well-focused hypothesis-driven studies.

9.6 FORCE HEALTH PROTECTION RESEARCH, 2003+

The Defense Science Board recommended in June 1994 that there was insufficient evidence to support the concept of a new syndrome and that many veterans' symptom reports were similar to chronic fatigue syndrome. At the end of a decade of research into Gulf War Illnesses, this assessment has held up despite examination of virtually every testable hypothesis, no matter how unlikely or unpopular. In the course of this research effort, many related medical issues were solved or knowledge was markedly advanced, and the DoD has adopted a new emphasis on pre- and post-deployment health issues. Joshua Lederberg, the chairman of the task force on Persian Gulf War Health Effects, noted that "***high-tech, low-casualty campaigns in exotic places will engender a preoccupation with residual health effects as a fact of life for the foreseeable future. If chemical or biological weapons are ever actually employed, there will be a gross multiplication of those residuals (on top of obvious acute physical and psychological casualties), and further research is needed on long-term consequences of exposure.***" The panel reiterated the need for better pre- and post-deployment medical assessments coordinated between DoD and VA. The current focus of the U.S. Army Medical and Materiel Command (USAMRMC) Force Health Protection (FHP) research program is shown in Table 9-3.

Table 9-3: Current Force Health Protection (FHP) Research, Showing Examples of Research Initiatives

- Identify fitness components affecting mission readiness
- Explore neuroprotective benefits of exercise
- Develop strategies to improve weight management in military environments
- Determine factors affecting effectiveness of educational interventions in basic training for health damaging behaviours
- Develop more effective psychological support systems for deployed family members and families
- Examine jet fuel exposure and neurological health in military personnel
- Assess permethrin exposure in operational conditions
- Monitor uncontrolled exposures through longitudinal prospective examination of military members (Millenium Cohort Study)
- Retrospectively study military occupation and neurodegenerative diseases
- Develop system to link surveillance data to health and fitness data
- Develop data mining capabilities for health outcomes derived from military electronic health records
- Develop a robust validated assessment battery to track neuropsychological health status of soldiers
- Assess neurocognition prospectively in future Gulf-deployed and Gulf-non-deployed soldiers

In 1999, three DoD Centers for deployment health were established to fulfill key roles in protection, detection, and treatment of disease threats in future deployments (Table 9-4).

Table 9-4: DoD Centers for Deployment Health

- **Research**: Center for Deployment Health Research (Naval Health Research Center, San Diego, California) (http://www.nhrc.navy.mil/rsch/department164/program.htm)
- **Surveillance**: Defense Health Surveillance Center (Defense Medical Surveillance System, Center for Health Promotion and Preventive Medicine, Aberdeen Proving Grounds, Maryland) (http://amsa.army.mil/)
- **Treatment**: Deployment Health Clinical Center (Gulf War Health Center, Walter Reed Army Medical Center, Washington, D.C.) (http://www.pdhealth.mil/)

The Center for Deployment Health Research includes the Millenium Cohort (MilCohort) study in its portfolio of center projects [100]. The MilCohort is a 21-year study of a stratified cohort of nearly 80,000 service members enrolled in 2001 (before the September 2001 attacks on the World Trade Center and the Pentagon) [101]. Two additional enrollment periods in 2004 (30,000 more enrollees) and 2007 expand the group to provide data on secular (temporal) trends. Data collection includes self-report surveys of the cohort every three years, combined with data from other records on occupational exposures, deployments, injury, medications, health care utilization, disability, and mortality. The participants are being followed beyond their periods of military service. One of the first reports on neurological health focused on mental health status of service members after the September 11 terrorist attacks [102].

The data emerging from recent studies indicate the important interactive roles of psychological stress and neurotoxic chemical exposures. For soldiers returning from recent deployments in Afghanistan and Iraq, the primary concerns are about psychological stress and mild head trauma from impact or blast, distinguishing these two causes and examining their interactions to produce psychiatric casualties and longer term neurological sequalae. One of the needs identified out of the Gulf War experiences is a clearly defined neuropsychological baseline test. This has been developed with support from the Gulf War Illnesses research program, Force Health Protection research program, and the Neurotoxin Exposure Treatment Research Program. One recent study demonstrated changes during deployment that may reflect normal brain plasticity in response to the requirements of the environment, in this case with deployment to Iraq, a hypervigilance requirement during operations and persisting post-deployment may be the explanation for a post deployment increase in reaction time as well as a decrease in some other cognitive functions such as short term memory [103]. Further studies that include testing with neuroimaging and biochemical markers, as well as pathological changes in neurodegenerative diseases will continue to refine the testing and monitoring capability for neurocognitive function, a capability that did not exist during the first Gulf War.

9.7 CONCLUSIONS

In conclusion, the U.S. DoD has invested a large amount of resources to investigate undiagnosed Gulf War Illnesses (GWI). Much of this expenditure could be avoided in future deployments with better characterization of exposures, pre-deployment investigation of health risks associated with likely neurotoxic exposures, and better tools to monitor exposures and health of individuals (i.e. biomarkers of toxic hazards). The Gulf War

Illnesses research produced important new understanding of some of the relevant post-deployment health issues and potentially hazardous occupational, materiel, and environmental exposures. Gulf War Illnesses issues also created a new awareness of important neuropsychological and neurotoxicological interactions which were not new problems but which represented a difficult and relatively untapped frontier in biomedical research in chronic multi-symptom illnesses. Some GWI topics such as blood-brain barrier integrity during stressful conditions and the neurological effects of depleted uranium have been addressed, but others such as the neuroprotective benefits of aerobic exercise and psychosocial influences on individual stress resilience and resistance to neurotoxicity remain important areas of investigation.

9.8 REFERENCES

[1] Fukuda, K., Nisenbaum, R., Stewart, G., Thompson, W.W., Robin, L., Washko, R.M., Noah, D.L., Barrett, D.H., Randall, B., Herwaldt, B.L., Mawle, A.C. and Reeves, W.C. Chronic multisymptom disease affecting Air Force veterans of the Gulf War. JAMA 1998; 280:981-988.

[2] Kang, H. and Bullman, T.A. Mortality among U.S. veterans of the Persian Gulf War. N England J Med 1996; 335:1498-1504.

[3] Kang, H. and Bullman, T.A. Mortality among U.S. veterans of the Persian Gulf War: 7-year follow-up. Am J Epidemiol 2001; 154:399-405.

[4] Hyams, K.C., Wignall, F.S. and Roswell, R. War syndromes and their evaluation: From the U.S. Civil War to the Persian Gulf War. Ann Intern Med 1996; 125:398-405.

[5] Clauw, D. The health consequences of the first Gulf War – the lessons are general (and for many patients) rather than specific to that war. BMJ 2003; 327:1357-8.

[6] Marlowe, D.H. and Norwood, A.E. Somatic consequences and symptomatic responses to stress: directions for future research. Technical Report. NTIS A170473. Bethesda MD; Uniformed Services University of the Health Sciences, 1999.

[7] Kirmayer, L.J., Young, A. and Robbins, J.M. Symptom attribution in cultural perspective. Can J Psychiatry 1994; 39:584-595.

[8] NIH Technology Assessment Workshop Panel. The Persian Gulf experience and health. JAMA 1994; 272:391-396.

[9] Task Force on Persian Gulf War Health Effects. Defense Science Board. Report of the Defense Science Board Task Force on Persian Gulf War Health Effects. Washington D.C., Office of the Undersecretary of Defense for Acquisition and Technology, 1994.

[10] Joellenbeck, L.M. and Hernandez, L. The Institute of Medicine's independent scientific assessment of Gulf War health issues. Mil Med 2002; 167:186-90.

[11] Institute of Medicine. Report of the Committee to Review the Health Consequences of Service During the Persian Gulf War. Washington, D.C.; Institute of Medicine, 1996.

[12] Presidential Advisory Committee on Gulf War Veterans' Illnesses. Final Report. Washington DC: U.S. Government Printing Office, 1997.

[13] http://govinfo.library.unt.edu/oversight/report_special.html (Last accessed 6 February 2007).

[14] Available online at: http://www.rand.org/multi/gulfwar/publications.html (Last accessed 6 February 2007).

[15] http://www.research.va.gov/resources/pubs/GulfWarRpt05.cfm (Last accessed 6 February 2007).

[16] Joseph, S.C. A comprehensive clinical evaluation of 20,000 Persian Gulf War veterans. Comprehensive clinical evaluation program evaluation team. Mil Med 1997; 162:149-155.

[17] Roy, M.J., Koslowe, P.A., Kroenke, K. and Magruder, C. Signs, symptoms, and ill-defined conditions in Persian Gulf War veterans: findings from the comprehensive clinical evaluation program. Psychosom Med 1998; 60:663-668.

[18] Kroenke, K., Koslowe, P. and Roy, M. Symptoms in 18,495 Persian Gulf War veterans: latency of onset and lack of association with self-reported exposures. J Occ Environ Med 1998; 40:520-528.

[19] Martin, S., Gamel, J., Jackson, J., Aronson, N., Gupta, R., Rowton, E., Perich, M., McEvoy, P., Berman, J., Magill, A. and Hoke, C. Leishmaniasis in the United States military. Mil Med 1998; 12:801-7.

[20] Magill, A.J., Grogl, M., Gasser, R.A. Jr., Sun, W. and Oster, C.N. Visceral infection caused by Leishmania tropica in veterans of Operation Desert Storm. N England J Med 1993; 328:1383-1387.

[21] Martin, S.K., Thuita-Harun, L., Adoyo-Adoyo, M. and Wasunna, K.M. A diagnostic ELISA for visceral leishmaniasis, based on antigen from media conditioned by Leishmania donovani promastigotes. Ann Tropical Med Parasitol 1998; 92:571-7.

[22] Nicolson, G.L. and Nicholson, N.L. Diagnosis and treatment of mycoplasmal infections in Persian Gulf War illnesses-CFIDS patient. International Journal of Occupational Medicine Immunology and Toxicology 1996; 5:69-78.

[23] AIBS. Letter Review to the U.S. Army Medical Research and Materiel Command. Peer Review to USAMRMC. Etiology of Gulf War Illness (Mycoplasma) – Final Report-February 26, 2001. Washington, D.C.; AIBS, 2001.

[24] Hyman, E.S. A urinary marker for occult systemic coccal disease. Nephron 1994; 68:314-326.

[25] Donta, S.T., Engel, C.C., Collins, J.F., Baseman, J.B., Dever, L.L. and Taylor, T., et al. Benefits and harms of doxycycline treatment for Gulf War veterans' illnesses. Ann Intern Med 2004; 141:85-94.

[26] Burkman, K.D., Moore, G.E. and Peterson, M.R. Incidence of zoonotic diseases in military working dogs serving in Operations Desert Shield and Desert Storm. Mil Med 2001; 166:108-111.

[27] McDiarmid, M.A., Keogh, J.P. and Hooper, F.J., et al. Health effects of depleted uranium on exposed Gulf War veterans. Environ Res 2000; 82:168-180.

[28] McDiarmid, M.A., Squibb, K., Engelhardt, S., Oliver, M., Gucer, P., Wilson, P.D., Kane, R., Kabat, M., Kaup, B., Anderson, L., Hoover, D., Brown, L. and Jacobson-Kram, D. Surveillance of depleted uranium exposed Gulf War veterans: health effects observed in an enlarged "friendly fire" cohort. J Occ Environ Med 2001; 43:991-1000.

[29] Hahn, F.F., Guilmette, R.A. and Hoover, M.D. Implanted depleted uranium fragments cause soft tissue sarcomas in the muscles of rats. Environ Health Perspec 2002; 110:51-59.

[30] Barber, D.S., Ehrich, M.F. and Jortner, B.S. The effect of stress on the temporal and regional distribution of uranium in rat brain after acute uranyl acetate exposure. J Toxicol Envrion Health 2005; 68:99-111.

[31] The Royal Society. The health effects of depleted uranium munitions. (Document 6/02). London, U.K.: Royal Society, 2002. Available online at: http://www.royalsoc.ac.uk/landing.asp?id=1243 (Last accessed 7 February 2007).

[32] Durakovic, A. Undiagnosed illnesses and radioactive warfare. Croat Med J 2003; 44:520-532.

[33] Araneta, M.R., Moore, C.A., Olney, R.S., Edmonds, L.D., Karcher, J.A., McDonough, C., Hiliopoulos, K.M., Schlangen, K.M. and Gray, G.C. Goldenhar syndrome among infants born in military hospitals to Gulf War veterans. Teratology 1997; 56:244-251.

[34] Cowan, D.N., Defraites, R.F., Gray, G.C., Goldenbaum, M.B. and Wishik, S.M. The risk of birth defects among children of Persian Gulf War veterans. N England J Med 1997; 336:1650-1656.

[35] Araneta, M.R., Destiche, D.A., Schangen, K.M., Merz, R.D., Forrester, M.B. and Gray, G.C. Birth defects prevalence among infants of Persian Gulf War veterans born in Hawaii, 1989-1993. Teratology 2000; 62:195-204.

[36] Schrader, S.M., Langford, R.E., Turner, T.W., Breitenstein, M.J., Clark, J.C., Jenkins, B.L., Lundy, D.O., Simon, S.D. and Weyandt, T.B. Reproductive function in relation to duty assignments among military personnel. Reproductive Toxicology 1998; 12:465-468.

[37] Araneta, M.R., Kamens, D.R., Zau, A.C., Gastanaga, V.M., Schlangen, K.M., Hiliopoulos, K.M. and Gray, G.C. Conception and pregnancy during the Persian Gulf War: the risk to women veterans. Ann Epidemiol 2004; 14:109-116.

[38] Levine, P.H., Young, H.A., Simmens, S.J., Rentz, D., Kofie, V.E., Mahan, C.M. and Kang, H.K. Is testicular cancer related to Gulf War deployment? Evidence from a pilot population-based study of Gulf War era veterans and cancer registries. Mil Med 2005; 170:149-153.

[39] Knoke, J.D., Gray, G.C. and Garland, F.C. Testicular cancer and Persian Gulf War service. Epidemiology 1998; 9:648-653.

[40] Bernstein, J.A., Perez, A., Floyd, R. and Bernstein, I.L. Is burning semen syndrome a variant form of seminal plasma hypersensitivity? Obstet Gynecol 2003; 101:93-102.

[41] Naval Health Research Center, http://www.nhrc.navy.mil/rsch/department164/projects/birthdefects.htm (Last accessed 7 February 2007).

[42] Cook, M.R., Graham, C., Sastre, A. and Gerkovich, M.M. Physiological and performance effects of pyridostigmine bromide in healthy volunteers: a dose-response study. Psychopharmacology 2002; 162:186-192.

[43] Somani, S.M., Husain, K., Asha, T. and Helfert, R. Interactive and delayed effects of pyridostigmine and physical stress on biochemical and histological changes in peripheral tissues of mice. J Appl Toxicol 2000; 20:327-334.

[44] Loewenstein-Lichtenstein, Y., Schwarz, M., Glick, D., Norgaard-Pedersen, B., Zakut, H. and Soreq, H. Genetic predisposition to adverse consequences of anticholinesterases in atypical BCHE carriers. Nat Med 1995; 1:1082-1085.

[45] Olveiera, G.H., Henderson, J.D. and Wilson, B.W. Cholinesterase measurements with an automated kit. Am J Ind Med 2002; Suppl 2:49-53.

[46] Wilson, B.W., Henderson, J.D., Ramirez, A. and O'Malley, M.A. Standardization of clinical cholinesterase measurements. Int J Toxicol 2002; 21:385-388.

[47] Gordon, R.K., Haigh, J.R., Garcia, G.E., Feaster, S.R., Riel, M.A., Lenz, D.E., Aisen, P.S. and Doctor, B.P. Oral administration of pyridostigmine bromide and huperzine A protects human whole blood cholinesterases from ex vivo exposure to soman. Chemical and Biological Interactions 2005; 157:239-246.

[48] Friedman, A., Kaufer, D., Shemer, J., Hendler, I., Soreq, H. and Tur-Kaspa, I. Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response. Nat Med 1996; 2:1382-1385.

[49] Kaufer, D., Friedman, A., Seidman, S. and Soreq, H. Acute stress facilitates long-lasting changes in cholinergic gene expression. Nature 1998; 393:373-377.

[50] Pope, C. Organophosphorus pesticides: do they all have the same mechanism of toxicity? J Toxicol Environ Health Part B: Critical Reviews 1999; 2:161-181.

[51] Usmani, K.A., Rose, R.L. and Hodgson, E. Inhibition and activation of the human liver microsomal and human cytochrome P450 3A4 metabolism of testosterone by deployment-related chemicals. Drug Metab Dispos 2003; 31:384-391.

[52] Song, X., Tian, H., Bressler, J., Pruett, S. and Pope, C. Acute and repeated restraint stress have little effect on pyridostigmine toxicity or brain regional cholinesterase inhibition in rats. Toxicol Sci 2002; 69:157-164.

[53] Abou-Donia, M., Wilmarth, K.R., Jensen, K.F., Oehme, F.W. and Kurt, T.L. Neurotoxicity resulting from coexposure to pyridostigmine bromide, DEET, and permethrin: implications of Gulf War chemical exposures. J Toxicol Environ Health Part A 1996; 48:35-56.

[54] Baynes, R.E., Halling, K.B. and Riviere, J.E. The influence of diethyl-,-toluamide (DEET) on the percutaneous absorption of permethrin and carbaryl. Toxicol App Pharmacol 1997; 144:332-339.

[55] Haley, R.W. and Kurt, T.L. Self-reported exposure to neurotoxic chemical combinations in the Gulf War. A cross-sectional epidemiologic study. JAMA 1997; 277:231-237.

[56] Rossi, et al, unpublished data 1997.

[57] Nordholm, A.F., Rossi, III, J., Ritchie, G.D., McInturf, S. and Hulme, M.E., et al. J Toxicol Environ Health 1999; 56:471-499.

[58] Roy, M.J., Kraus, P.L., Seegers, C.A., Young, S.Y., Kamens, D.R. and Law, W.A., et al. Pyridostigmine, diethyltoluamide, permethrin, and stress: a double-blind, randomized, placebo-controlled trial to assess safety. Mayo Clin Proc 2006; 81:1303-1310.

[59] Proctor, S.P., Heeren, T., White, R.F., Wolfe, J., Borgos, M.S., Davis, J.D., Pepper, L., Clapp, R., Stuker, P.B., Vasterling, J.J. and Oznoff, D. Health status of Persian Gulf War veterans: Self-reported symptoms, environmental exposures and the effect of stress. Int J Epidemiol 1998; 27:1000-1010.

[60] Cheng, Y.-S., Zhou, Y., Chow, J., Watson, J. and Frazier, C. Chemical composition of aerosols from kerosene heaters burning jet fuels. Aerosol Sci Technol 2001; 35:949-957.

[61] Doebbeling, B.N., Heller, J.M., Lange, J.L., Schwartz, D.A. and Thorne, P.S. Exposures to the Kuwait oil fires and their association with asthma and bronchitis among Gulf War veterans. Environ Health Perspec 2002; 110:1141-1146.

[62] Smith, T., Heller, J.M., Hooper, T.I. and Gackstetter, G. Are Gulf War veterans experiencing illness due to exposure to smoke from Kuwaiti oil well fires: Examination of Department of Defense hospitalization data. Am J Epidemiol 2002; 155:908-917.

[63] Committee on Toxicology. National Research Council. Toxicological Assessment of Jet-Propulsion Fuel 8. Washington D.C.; The National Academies Press 2003.

[64] Conn, C.A., Dokladny, K. and Menache, M.G., et al. Effects of sarin on temperature and activity of rats as a model for Gulf War Syndrome neuroregulatory functions. Toxicol Appl Pharmacol 2002; 184:77-81.

[65] Henderson, R.F., Barr, E.B., Blackwell, W.B., Clark, C.R., Conn, C.A., Kalra, R., March, T.H., Sapori, M.L., Tesfaigzi, Y., Menache, M.G. and Mash, D.C. Response of rats to low levels of sarin. Toxicol Appl Pharmacol 2002; 184:67-76.

[66] Van Helden, H.P., Vanwersch, R.A., Kuijpders, W.C., Trap, H.C., Phillippens, I.H. and Benschop, H.P. Low levels of sarin effect the EEG in marmoset monkeys: a pilot study. J Appl Toxicol 2004; 24:475-83.

[67] Page, W.F. Long-term health effects of exposure to sarin and other anticholinesterase chemical warfare agents. Mil Med 2003; 168:239-245.

[68] Bullman, T.A., Mahan, C.M., Kang, H.K. and Page, W.F. Mortality in U.S. Army Gulf War veterans exposed to 1991 Khamisiyah chemical munitions destruction. Am J Public Health 2005; 95:1382-1388.

[69] Miller, R.N., Costigan, D.J., Young, H.A., Kang, H.K., Dalager, N., Mathes, R.W., Crawford, H.C., Page, W.F. and Thaul, S. Patterns of health care seeking of Gulf War registry members prior to deployment. Mil Med 2006; 171:370-375.

[70] Haley, R.W., Billecke, S. and La Du, B.N. Association of low PON1 type Q (type A) arylesterase activity with neurologic symptom complexes in Gulf War veterans. Toxicol Appl Pharmacol 1999; 157:227-233.

[71] Joellenbeck, L.M., Zwanziger, L.L., Durch, J.S. and Strom, B.L. (Eds). The Anthrax Vaccine. Is it Safe?: Does it Work? Washington DC; National Academy Press, 2002.

[72] Hotopf, M., David, A., Hull, L., Ismail, K., Unwin, C. and Wessely, S. Role of vaccinations as risk factors for ill health in veterans of the Gulf war: cross sectional study. BMJ 2000; 320:1363-1367.

[73] Peakman, M., Skowera, A. and Hotopf, M. Immunological dysfunction, vaccination and Gulf War illness. Philos Trans R Soc Lond B Biol Sci 2006; 361:681-687.

[74] Asa, P.B., Cao, Y. and Garry, R.F. Antibodies to squalene in Gulf War Syndrome. Exp Mol Pathol 2000; 68:55-64.

[75] Horner, R.D., Kamins, K.G., Feussner, J.R., Grambow, S.C., Hoff-Lindquist, J., Harati, Y., Mitsumoto, H., Pascuzzi, R., Spencer, P.S., Tim, R., Howard, D., Smith, T.C., Ryan, M.A., Coffman, C.J. and Kasarskis, E.J. Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. Neurology 2003; 61:742-749.

[76] Haley, R.W. Excess incidence of ALS in young Gulf War veterans. Neurology 2003; 61:750-756.

[77] Haley, R.W., Fleckensteinm J.L., Marshall, W.W., McDonald, G.G., Kramer, G.L. and Petty, F. Effect of basal ganglia injury on central dopamine activity in Gulf War Syndrome: Correlation of proton magnetic resonance spectroscopy and plasma homovanillic acid levels. Arch Neurol 2000; 57:1280-1285.

[78] Weisskopf, M.G., O'Reilly, E.J., McCullough, M.L., Calle, E.E., Thun, M.J., Cudkowicz and Ascherio, A. Prospective study of military service and mortality from ALS. Neurology 2005; 64:32-37.

[79] DeFraites, R.F., Wanat, E.R., Norwood, A.E., Williams, S. and Cowan, D. Investigation of a Suspected Outbreak of an Unknown Disease among Veterans of Operation Desert Shield/Storm. 123d Army Reserve Command, Fort Benjamin Harrison, Indiana, April 1992. Technical Report WRAIR/R-06-0002. Washington, D.C.; Walter Reed Army Institute of Research, 1995.

[80] Gray, G.C., Coate, B.D., Anderson, C.M., Kang, H.K., Berg, S.W., Wignall, F.S., Knoke, J.D. and Barrett-Connor, E. The postwar hospitalization experience of U.S. veterans of the Persian Gulf War. N England J Med 1996; 335:1505-1513.

[81] Stretch, R.H., Bliese, P.D., Marlowe, D.H., Wright, K.M., Knudson, K.H. and Hoover, C.H. Physical health symptomatology of Gulf War-era service personnel from the states of Pennsylvania and Hawaii. Mil Med 1995; 160:131-136.

[82] Storzbach, D., Campbell, K.A., Binder, L.M., McCauley, L., Anger, W.K., Rohlman, D.S. and Kovera, C.A. Psychological differences between veterans with and without Gulf War unexplained symptoms. Portland Environmental Hazards Research Center. Psychosom Med 2000; 62:726-735.

[83] Gray, G.C., Kaiser, K.S., Hawksworth, A.W., Hall, F.W. and Barrett-Connor, E. Increased postwar symptoms and psychological morbidity among U.S. Navy Gulf War veterans. Am J Trop Med Hyg 1999; 60:758-766.

[84] Eisen, S.A., Kang, H.K. and Murphy, F.M., et al. Gulf War veterans' health: medical evaluation of a U.S. cohort. Ann Intern Med 2005; 142:881-890.

[85] Doebbeling, B.N., Clarke, W.R., Watson, D., Torner, J.C., Woolson, R.F. and Voelker, M.D, et al. Is there a Persian Gulf War syndrome? Evidence from a large population-based survey of veterans and nondeployed controls. Am J Med 2000; 108:695-704.

[86] Unwin, C., Blatchley, N., Coker, W., Ferry, S., Hotopf, M., Hull, L., Ismail, K., Palmer, I., David, A. and Wessely, S. Health of UK servicemen who served in Persian Gulf War. Lancet 1999; 353:169-178.

[87] Haley, R.W., Kurt, T.L. and Hom, J. Is there a Gulf War syndrome? Searching for syndromes by factor analysis of symptoms. JAMA 1997; 277:215-222.

[88] Haley, R.W., Hom, J., Roland, P.S., Bryan, W.W., Van Ness, P.C., Bonte, Sr., F.J., Mathews, D., Fleckenstein, J.L., Wians, F.H., Wolfe, G.I. and Kurt, T.L. Evaluation of neurologic function in Gulf War veterans. A blinded case-control study. JAMA 1997; 277:223-230.

[89] The Research Advisory Committee to the Department of Veterans' Affairs on Gulf War Veterans' Illnesses. 2004 Report and Recommendations. Washington, D.C.; Veteran's Administration, 2004.

[90] Couzin, J. Veterans Administration. Texas earmark allots millions to disputed theory of Gulf War illness. Science 2006; 312:668.

[91] Hotopf, M., David, A.S., Hull, L., Nikalaou, V., Unwin, C. and Wessely, S. Gulf war illness – better, worse, or just the same? A cohort study. BMJ 2006; 327:1370-1373.

[92] Kipen, H.M., Hallman, W., Kang, H., Fiedler, N. and Natelson, B.H. Prevalence of chronic fatigue and chemical sensitivities in Gulf Registry veterans. Arch Environ Health 1999; 54:313-318.

[93] Donta, S.T., Clauw. D.J. and Engel, C.C., et al. Cognitive behavioural therapy and aerobic exercise for Gulf War veteran's illnesses. JAMA 2003; 289:1396-1404.

[94] Lucas, K.E., Armenian, H.K., Debusk, K., Calkins, H.G. and Rowe, P.C. Characterizing Gulf War illnesses: neurally mediated hypotension and postural tachycardia syndrome. Am J Med 2005; 118:1421-1427.

[95] Spencer, P.S., McCauley, L.A., Lapidus, J.A., Lasarev, M., Joos, S.K. and Storzbach, D. Self-reported exposures and their association with unexplained illness in a population-based case-control study of Gulf War veterans. J Occup Environ Med 2001; 43:1041-1056.

[96] Sorg, B.A. and Bell, I.R. (eds.). The Role of Neural Plasticity in Chemical Intolerance. Ann N Y Acad Sci 2001; 933:1-329.

[97] Dalton, P. Cognitive influences on health symptoms from acute chemical exposure. Health Psychol 1999; 18:579-590.

[98] Fiedler, N., Giardino, N., Natelson, B., Ottenweller, J.E., Weisel, C., Lioy, P., Lehrer, P., Ohman-Strickland, P., Kelly-McNeil, K. and Kipen, H. Responses to controlled diesel vapour exposure among chemically sensitive Gulf War veterans. Psychosomatic Medicine 2004; 66:588-598.

[99] Cohen, G. Oxidative stress, mitochondrial respiration, and Parkinson's Disease. Annals of the New York Academy of Sciences 2000; 899:112-120.

[100] http://www.millenniumcohort.org/ (Last accessed 8 February 2007).

[101] Chesbrough, K.B., Ryan, M.A.K., Amoroso, P., Boyko, E.J., Gackstetter, G.D., Hooper, T.I., Riddle, J.R. and Gray, G.C. The Millenium Cohort Study: a 21-year prospective cohort study of 140,000 military personnel. Mil Med 2002; 167:483-8.

[102] Smith, T.C., Smith, B., Corbeil, T.E., Riddle, J.R. and Ryan, M.A. Self-reported mental health among U.S. military personnel prior and subsequent to the terrorist attacks of September 11, 2001. J Occup Environ Med 2004; 46:775-782.

[103] Vasterling, J.J., Proctor, S.P., Amoroso, P., Kane, R., Heeren, T. and White, R.F. Neuropsychological outcomes of army personnel following deployment to the Iraq war. JAMA 2006; 296:519-529.

Chapter 10 – HEALTH RISKS DURING THE LIFE CYCLE OF THE DEPLOYED SOLDIER

by

Christian Carton[1], Karl Friedl[2], Birgitta Liljedahl[3], David Lam[4]

[1]Service of Preventive Medicine, Belgian Defence, Brussels, Belgium

[2]U.S. Army Medical Research and Materiel Command, Fort Detrick, Maryland, U.S.A.

[3]Division of NBC Defence, Swedish Defence Research Agency (FOI-skydd), Umea, Sweden

[4] U.S. Army Telemedicine and Advanced Technology Research Center, Ft. Detrick, MD, U.S.A. and University of Maryland Medical School, Baltimore, Maryland, U.S.A.

10.1 ABSTRACT

This chapter describes the timely management of occupational health risks during the entire life cycle of a soldier, from first selection as a recruit to beyond the end of his or her military career. The goal of military occupational and environmental health (OEH) programs is to contribute to the operational availability and capability of the military personnel as well as to protect their long-range health and general well-being. The monitoring of toxic hazards is especially important in OEH programs not only for potential toxic exposure itself, but because of potential interactions of such toxins with virtually every other aspect of military health. Biomonitoring established with toxic hazards in mind offers strategies for health monitoring that are likely to be useful for other health risks and outcomes as well. In a military career there are four stages: recruitment; training; operational deployment; and retirement. Health risk monitoring approaches for each of these stages are discussed.

10.2 INTRODUCTION

Occupational health standards and the management of health risks in a deployed military setting differ from most other occupations because of the deadly risks already inherent in warfighting occupations, with operational requirements not typically encountered in most other professions. The most important difference is the military operational need for short-term expedient exposures which in other occupational environments would be considered high risk, but which on an acute basis may actually be tolerable and necessary. Many of these health threats are predictable but continue to be intensively studied in military labs because of the infinite variations of these stressors that may confront service members (Figure 10-1). For example, the need to exploit hazardous environments of strategic importance may require tradeoffs between temporary exposure to potentially neurotoxic pesticides and unprotected exposure to malarial mosquitoes, or brief exposures to potentially hazardous environments, such as burning oil wells, that may be necessary during completion of a vital wartime task. There are many other special considerations in military health risk management ranging from combat materiel that may present health hazards to friendly forces (e.g. depleted uranium, low level chemical agents, laser rangefinders) to certain individual behaviors that may be more prevalent in risk takers and military environments (e.g. risky sexual behavior, physical fitness habits, smoking, alcohol abuse) that may substantially modify health risks in deployments. In addition to inadvertent or unavoidable risks in operational environments, the level of risk which may be acceptable in training environments may be determined by the need for realistic high intensity training for which there is no safer surrogate training model. Biomonitoring technologies are central to health risk management across the life cycle of the soldier (from recruitment to post-retirement) and can help to reduce the health risks by providing

better information to leaders so they can make better risk management decisions, conduct better health monitoring, establish better protective measures, and be provided better options for courses of action in risk environments.

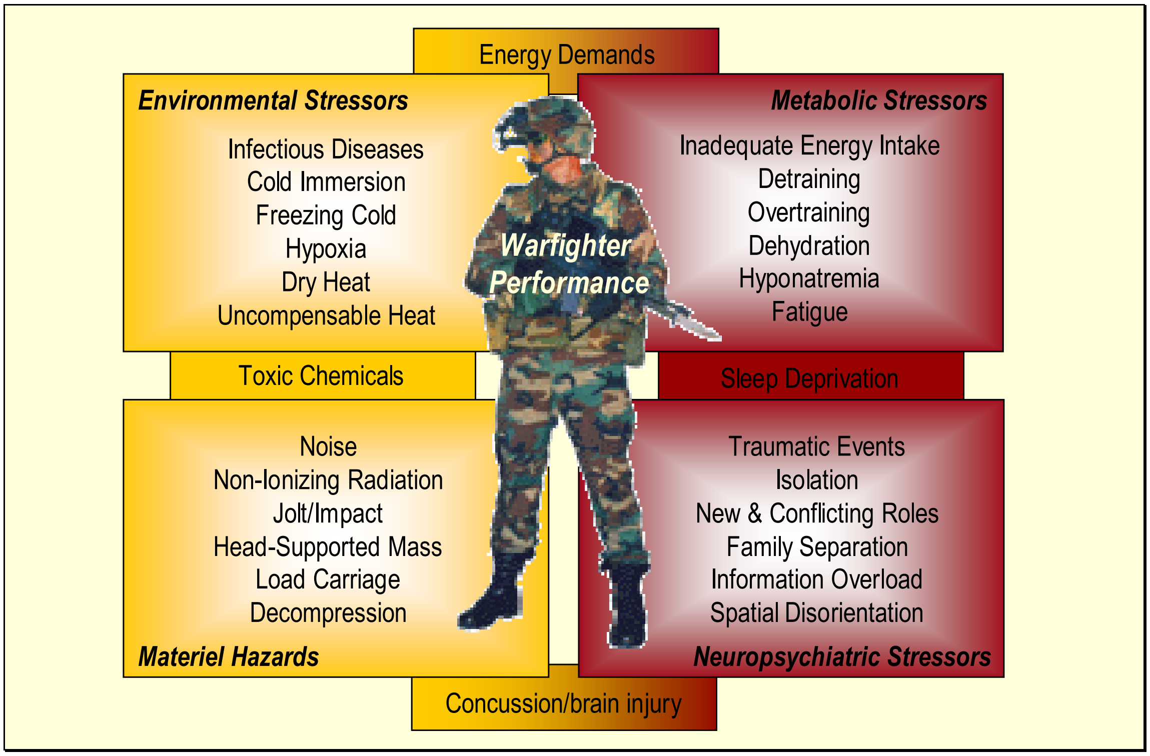

Figure 10-1: Predictable Stressors Confront Warfighters During Training and in Deployments. These have been studied individually and in combination, with continued research on predictive models, monitoring strategies and countermeasures. New variations of these stressors are often encountered.

The medical standards of fitness that determine who will enter the military and the allowable tasks to which an individual may be assigned are critical to preventing avoidable injury and illnesses. The training phase is both an opportunity for health promotion/protection and a new set of health risks. Individual resilience may be enhanced by effective training. Health risks may also be increased for some individuals in the attempt to provide a realistic high intensity training experience. Health care expertise is needed to forecast and control the occupational health risks in deployment with the goal of minimizing avoidable morbidity and mortality. In operations the soldier might be confronted with specific hazards. These hazards have to be identified as early in the planning cycle as is possible, and the associated risks have to be assessed. What is the likelihood of harmful incidents occurring and their potential injury and illness consequences? For each combination of hazard and disease an appropriate monitoring strategy including a specific health surveillance has to be established. These risks may range from those experienced only by susceptible subgroups such as pregnant soldiers or individuals with a genetic predisposition to injury (e.g. G-6-PD deficient soldiers who may not be able to take certain anti-malarial drugs); to specialized exposure groups such as submariners living in closed air environments or aircraft refuelers; or to the entire force when a new chemical prophylaxis is used during deployment or camps are established near burning garbage dumps. There is more to fitness and health than the absence of disease or injury. Occupational health interventions are required throughout the entire career of a

soldier to promote health and a healthy lifestyle, and thus to increase availability and operational capability. These same beneficial individual health behaviors may carry beyond military service in healthy veterans, thus encouraging longevity while decreasing medical expenditures. Alternatively, behaviors acquired in the military may produce a significant health care burden for the individual and the country if behaviors learned in the military degrade long-term health (e.g. cigarette smoking), if the sudden cessation of exercise and weight management habits leads to worse health consequences than for chronically sedentary overweight individuals, or if inappropriate and excessive physical training leads to an increased risk of osteoarthritis.

10.3 RECRUITMENT

Minimum baseline health and fitness standards have to be applied in order to be able to achieve the training standards. If a new recruit is not physically and psychiatrically suitable for military training, he or she will probably not complete the training, or will require much remedial training to meet standards. In either case, this has a significant adverse impact upon the training establishment, and may reduce the combat effectiveness of the force. Therefore at the time of recruitment it is important to give attention to the personal history: hereditary medical problems, habitual levels of physical activity, injuries and illnesses, mental health, psychosocial history, sexual development, history of depression and suicide, abuse, social support, risk behaviours, education, weight, nutrition, medication, dental care, chronic disease, etc. As an example, there is evidence that cigarette smoking is a predictor of general medical well-being during training [1]. A history of smoking could be regarded as a negative factor in the selection of recruits. Requiring and helping the recruits to stop smoking once they are inducted can help to achieve an improvement in their overall physical ability. There is also evidence that a higher level of physical activity or fitness before the recruitment helps to avoid training-related and time-loss injuries [2]. Therefore it is wise to require the candidates to meet well-defined minimum standards of fitness before induction.

Recruitment standards must be appropriately defined in order to minimize the likelihood of operationally-induced health hazards. Entry exams and history must both be generic enough to allow reasonably rapid accomplishment, and detailed enough to ensure that significant defects or disabilities are not missed. Follow-up tests, to include VO2, functional respiratory tests, neurological and psychological tests, etc. must be available for use in the evaluation of potential recruits whose ability to fully meet the standards is questionable. The validation against current mission requirements of these entry criteria is also important so that potentially excellent recruits are not being rejected for the wrong reasons. For example, the U.S. Army rejected many recruits from service in the past because of flat feet, a condition that was thought to be detrimental to health and performance of foot soldiers in training and on long marches. A careful study carried out by USARIEM and Nike in hundreds of basic trainees determined that flat feet were much less of a problem than the opposite, high arches [3]. As another example, up until World War 2, the U.S. Army rejected candidates who did not have two opposing (upper and lower) front teeth – upon investigation, it was determined that this requirement dated from before the Civil War, when it was necessary to have those teeth to bite open the paper cartridges then in use. Other seemingly well-established exclusion criteria such as attention deficit disorder and various forms of asthma have been periodically reconsidered. Medical standards that include body fat are intended to ensure healthy (i.e. non-morbidly obese) individuals, but these standards are also intended to ensure that new soldiers can later meet the medical standards for retention on active duty, which are more stringent. These retention standards include body fat standards that have been established to ensure individual combat readiness; lately these thresholds have been re-evaluated with pre-diabetes and other near term health and performance readiness consequences in mind [4].

In summary, the selection process should exclude those candidates with medical conditions which indicate a significant risk of them suffering ill health due to operational exposure or an inability to carry out military

functions. There should be a feedback loop from the lessons learned during operations to the medical centers responsible for recruitment, for instance when a significant ill health or disability related to a previously existing condition develops.

10.4 TRAINING CYCLES

The current and previously determined health status of the soldier should be checked by a medical staff member upon first arrival at a training center. Appropriate medical and/or administrative action should be taken to address any revelations and discoveries which might affect success in training or in operations. This may demand discharge from the service or medical care before the recruit is allowed to begin training. Gender-free training policy may provoke an increased rate of training injuries in female recruits [5]. Even at this early stage in their careers, Soldiers may be exposed to toxic hazards. A risk assessment of all potentially toxic substances used in training (cause of irritation, wounds, sensitisation, mutagenic effects, etc.) is necessary, and should be accomplished as part of the planning process for training events. Results of this assessment should be used in health awareness education during training. This education should not only cover matters such as personal cleanliness, smoking, alcohol, recreational drugs, reproductive health, sleep, diet and prevention of injuries and diseases, but also education about the prevention of thermal stress (e.g. dehydration), operational hygiene (personal protection and prevention), and methods of reducing the impact of potentially toxic substances.

It is never too early in a soldier's career for their inclusion in epidemiological surveillance systems. Data collected during routine clinical contacts and periodic health surveillance in a controlled environment can be very useful in later deployments when environmental and industrial risks cannot always be predicted and correctly evaluated. As an example, the importance of functional respiratory tests should be considered. The baseline values of FVC (Forced Vital Capacity), FEV1 (1 second Forced Expiratory Volume), and PEF (Peak Expiratory Flow) can be essential in the future evaluation of effects from exposure to airborne toxic substances such as chlorine, training gasses, phosgene, acids, fumes, and dust. Less is known about chemical agents causing bronchial hypersensitivity (e.g. acrylates, diisocyanates); normally the soldier should not be repeatedly exposed to these agents, but during operational deployments it cannot always be excluded or avoided. Sudden complaints of the respiratory function can only be objectively evaluated in comparison with a baseline value.

A similar case can be made for changes in mental functioning, with many neurotoxic chemical exposures that could be encountered in military environments affecting mood, cognition, psychomotor performance, and other neuropsychological domains. Detection of such changes requires baseline testing of soldiers upon entry into the military and periodic reassessments to adjust for ageing and other non-military exposure effects. Human studies indicate increased susceptibility to some illnesses such as the common cold in a "dose" association with semiquantitative stress burden [6]. Animal studies have shown increased toxicity of certain chemical compounds, such as malathion toxicity to tadpoles exposed to the psychological stress of predator scent in the water [7]. These are areas for further research before individual susceptibilities can be more definitely assessed and perhaps mitigated through resilience training.

Periodic health surveillance programs should try to cover all the physical, mental and psychological aspects essential for the well-being of the soldier. The rationale in the paragraphs above clearly argue in favor of extensive baseline functional studies being carried out on all soldiers as part of routine surveillance programs – significantly beyond the studies currently mandated in most NATO forces.

The U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM) has established military-specific exposure guidelines that attempt to take into account both immediate incapacitating effects that will affect the warfighter and mission success, and immediate, delayed or chronic health outcomes [8]. These values must be taken into account in planning training programs.

The U.S. Army uses a standardised formal process of health hazard assessment to evaluate all new materiel from the earliest possible stages of conception through the final fielding and use [9, 10]. For many of these evaluations of new types of equipment, new methods for assessing hazards (including toxic hazards) have to be developed. For example, safety of humans operating new high-powered weapons systems such as the Multiple Launch Rocket System (MLRS) required new standards and methods of assessing risk from blast overpressure and inhalation of toxic gases associated with systems, and for carrying out survivability assessments of defeated armor [11 – 13]. The safety of new permethrin impregnated personal equipment has required the development of new assays for permethrin metabolites and protein adducts. The research role in the process of health hazard assessment and survivability analyses is shown in Figure 10-2. In those instances in which risk evaluation standards and methods have not yet been developed, the use of new systems tends to be more limited by very conservative standards until new data is available to support more realistic and practical standards. Interim advice on safety may be provided on the basis of current data in advance of completion of a fully tested and validated method or standard, with the caveat that such advice will be frequently updated as new information is derived. This use of conservative interim standards pending final evaluation of hazard is critical so that the military can employ useful equipment and tactics when the best available science supports safe use, even if the final end point of hazard analysis has not yet been reached.

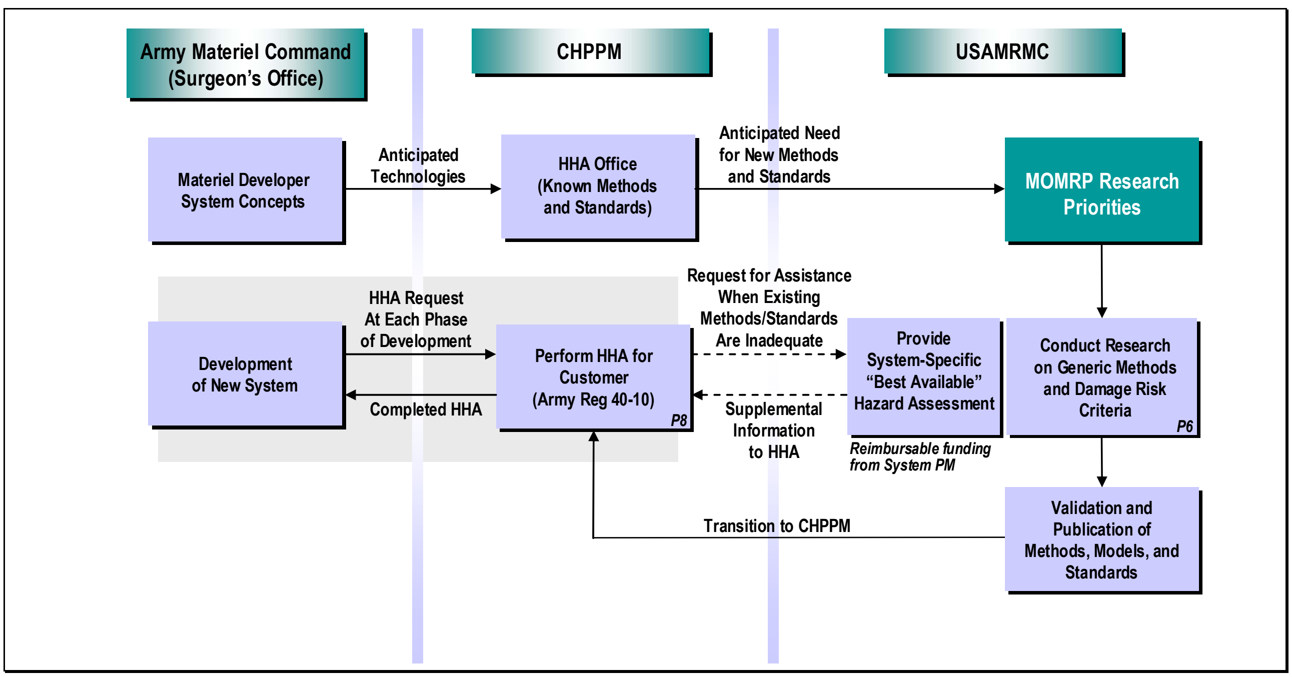

Figure 10-2: The Role of Research by the Military Operational Medicine Research Program (MOMRP) of the U.S. Army Medical Research and Materiel Command (USAMRMC) in Support of the Center for Health Promotion and Preventive Medicine (CHPPM) for the Health Hazard Assessment (HHA) Process Used by the US Army. New technologies typically present new health risks, include toxic hazards, and require conservative standards of health protection until new research establishes more realistic criteria and methods.

10.5 OPERATIONAL DEPLOYMENTS

A periodic clinical examination of the soldier is essential subsequent to initial entry examinations, even in the absence of clinical complaints or injuries. In addition to standard clinical indices, some armies also frequently assess physical fitness and body weight as part of such periodic evaluations. These measures can also provide early detection of changes in health status that suggest further evaluation. Clinical surveillance should include a set of complementary tests: hematological (RBC, WBC with differential analysis, Hemoglobin, Hematocrit); biochemical (hepatic and renal function); and health promotion screening (Blood Pressure, Serum Glucose, Cholesterol [including Total, HDL, and LDL]). These complementary tests are not only intended to evaluate the actual state of health, they should also help the soldier in aspects of health promotion and education such as in the prevention of cardiovascular diseases and diabetes. The early detection of diabetes or other cardiovascular risk factor is essential for the long-term well-being of the patient. Failure to monitor (and potentially prevent) development of such diseases might have important operational consequences, both individually and collectively.

Additional assessments and testing should be done in conjunction with deployments. Figure 10-3 highlights the variety of some of the health concerns typical of international deployments. Each deployment can bring about new and unpredictable health concerns involving psychological, infectious, toxic chemical, physical and other stressors. Assessment of standard clinical parameters is not likely to be adequate (or efficient) in pre- and post-deployment health testing for the wide variety of peculiar and unexpected new health hazards that may arise. This is to say that there is no certainty that all possible health issues will be addressed by any practical set of measures, except to ask soldiers about their health status and concerns, and then to be able to follow up on new or emerging threats through the analysis of collected environmental samples, blood samples, etc. Measurement of biochemical parameters in a stored serum sample certainly does not address all possible questions, contrary to common public perceptions (i.e. "why don't you just measure everything in a blood sample before and after a deployment to know that soldiers are still healthy?"). Biochemical markers are still elusive for many desired applications in toxicology outcomes, and are especially problematic in the evaluation of neurological and behavioural disorders. Thus, deployment testing should be designed to detect overall changes in health status, general health indicators, sampling collections for retrospective analyses, and some assessments that are tailored to potential specific classes of threats as determined by the nature of the deployment. For example, peacekeeping missions in the midst of civil conflict may require more than typical mental health assessments [14 – 15], while deployments into areas with high industrial and agricultural toxic chemical contamination may need to focus on toxicological markers and outcomes.

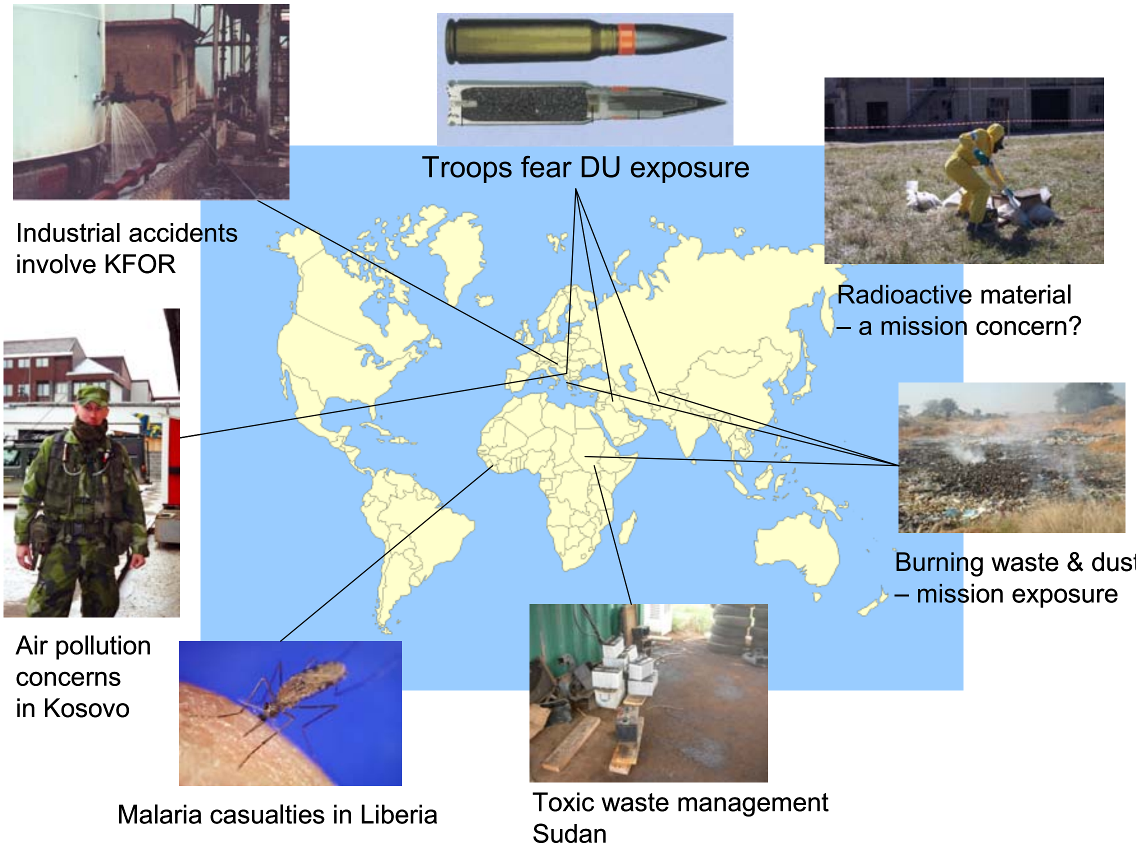

Figure 10-3: Examples of Recent Deployment Hazards Encountered by Peacekeeping Forces.

The relationship between health status and current military exposures in occupational and deployment environments is being systematically monitored in a sample of U.S. military members through the Millenium Cohort Study. This 21-year prospective study examines health outcomes of over 100,000 U.S. servicemembers starting with an initial sample enrolled in 2001 [16]. The study cohort was increased by an additional 30,000 members in 2004, and will add still more in 2007 to analyze temporal (generational) differences. Every three years, each member of the study is reassessed with an in-depth survey of recent exposures and deployment histories, as well as matching to other individual data such as inpatient and outpatient health care encounters. The participants are being followed beyond their periods of military service, and the Department of Veterans Affairs, also a participant, reviews records of all study subjects entering the VA system. A top priority for this project is to study potentially neurotoxic exposures and any associated neurological outcomes. The Millenium Cohort study has already been useful in assessing health status of service members before and after the World Trade Center bombing on Sept 11, 2001 [17].

A relatively new technology involving the combination of world surveillance databases provides a new tool for medical situational awareness and monitoring (Project Argus). A unique aspect of this database is the tracking of near-real time changes in social disruption as detected in translated news accounts and other definable events [18]. Thus, increasing reports in an area of the world of, for example, an increase in mysterious illnesses and hospitalizations, or large unexpected population movements, combined with various ground confirmation mechanisms may signify a chemical, biological, or radiological event or some natural

disaster, to include the emergence of previously unknown diseases. Further monitoring of the environment may provide additional insights from climate and other factors to predict disease outbreaks and other health threats in advance of exposure of deployed forces.

10.6 POST-MILITARY SERVICE

U.S. veterans have high rates of medical disability discharges from the Army for musculoskeletal conditions, and high-frequency sensory-neural hearing loss (HFSNHL) is endemic among military veterans. These are examples of potentially preventable problems related to some aspects of training, combat, or other military exposures. Careful studies of toxic chemical hazards such as petroleum product exposures and increased rates of noise induced hearing loss may reveal interactive effects in veterans associated with avoidable neurotoxic and noise exposures. These health outcomes are identified through epidemiological studies in combination with well-validated assessments of exposure and effect.

Behaviors supporting the maintenance of good health can be promoted in the military through various mechanisms, including exercise and weight management. One pending question is whether or not sudden cessation of these habits in new retirees precipitates rapid weight gain and loss of cardiovascular health and fitness, at rates higher than those who have remained sedentary and with less healthy eating habits for most of their life. For veterans maintaining their good health habits into retirement, there may be specific benefits to musculoskeletal and cardiovascular health later in life, including reduction of risks for osteoporosis, hypertension, sacropenia, and heart disease. Furthermore, remaining active with good habits learned during military service may prevent type 2 diabetes, neurodegenerative diseases, etc.

Military veterans are repositories of the accumulated toxic chemical and other harmful exposures over their lifetimes, and may serve as biosentinels for these effects in soldiers of the future. It may be possible to follow these veterans on a long-term basis, and to determine some effects specific to military service and militarily-unique exposures through well-designed population studies. There are several recent examples of studies that have been conducted post-hoc to determine effects of uncontrolled exposures. These include the effects of Agent Orange herbicide on U.S. veterans of the Vietnam war [19, 20]. A study of Japanese-Americans many of whom served in WWII includes assessment of brain pathology and chemical residues in dying ageing men [21]. A study of neurodegenerative diseases in military veterans compared to their peer groups indicated a higher rate of ALS but no difference in overall PD rate [22]. A comparison of groups from the 1970s that were used in experimental exposures to chemical agents versus soldiers used only in equipment tests, revealed no differences in health outcomes, although the test groups were not well matched because of non-random selection criteria in the initial studies [23]. Epidemiological studies of the causes of serious illness and death in specialized groups such as submariners may reveal something about inhalation risks associated with closed air environments on different types of submarines, although the appropriate control group for comparison of such a specialized group of largely self-selected individuals is a problem.

10.7 CONCLUSIONS

Figure 10-4 summarizes current monitoring and record keeping objectives that will help to quickly detect, identify, and prevent or reduce health risks in future deployments. Monitoring methods for toxic hazards are needed at every step. A proposed schema for standardisation of the identification of hazards through the implementation of control measures is shown in Figure 10-5.

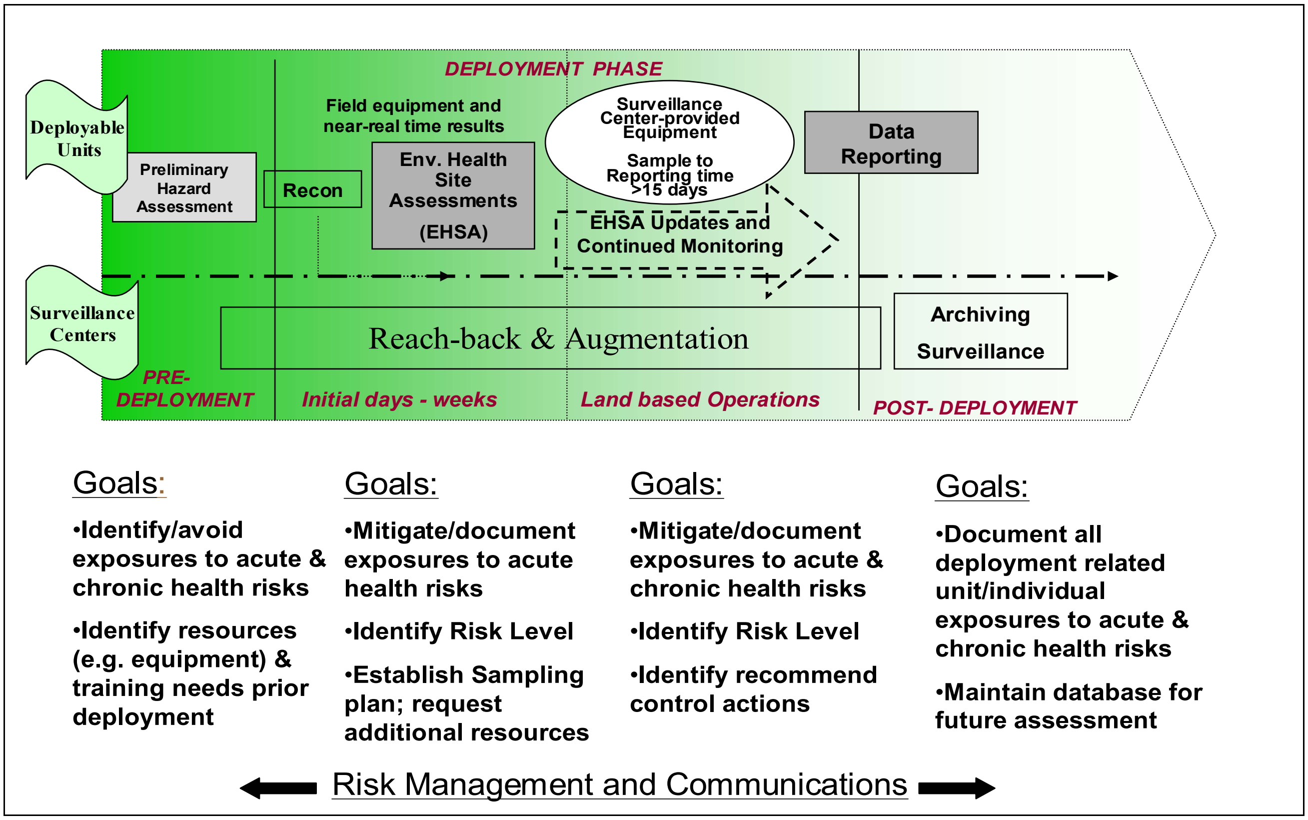

Figure 10-4: Overview of the U.S. DoD OEH Surveillance Process Including Steps During Pre-Deployment, Initial Deployment, Longer-Term Land-Based Operations, and Post-Deployment Phases (chart from presentation by COL Deniece Van Hook, presented at HFM-057/RTG-009 Gent meeting, 2006).

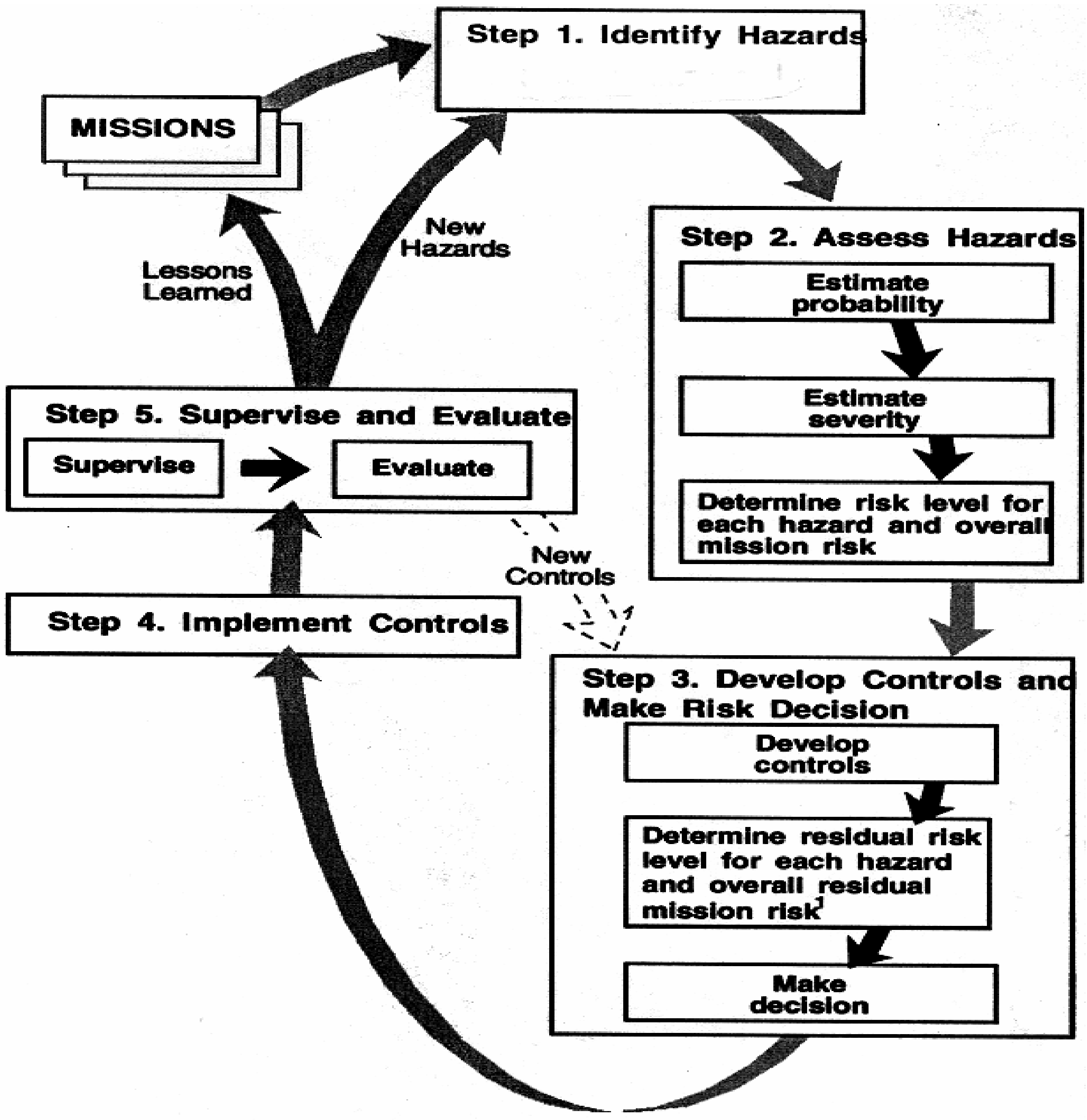

Figure 10-5: Health Risk Management Scheme Proposed for NATO. Steps 2 and 5 are key opportunities for the application of biomonitoring technologies [24].

10.8 REFERENCES

[1] Snoddy, R.O. Jr. and Henderson, J.M. Predictors of basic infantry training success. *Mil Med* 1994; 159:616-22.

[2] Knapik, J.J., Sharp, M.A., Canham-Chervak, M., Hauret, K., Patton, J.F. and Jones, B.H. Risk factors for training related injuries among men and women in basic combat training. *Med Sci Sports Exerc* 2001; 33:946-54.

[3] Cowan, D.N., Jones, B.H. and Robinson, J.R. Foot morphologic characteristics and risk of exercise-related injury. *Archives of Family Medicine* 1993; 2:773-777.

[4] Friedl, K.E. Can you be large and not obese? The distinction between body weight, body fat, and abdominal fat in occupational standards. *Diabetes Technology and Therapeutic*s 2004; 6:732-749.

[5] NATO DRG Panel VIII, *Workshop on optimising the performance of women in the Armed Forces of NATO*. London, United Kingdom; NATO RTO, 16-19 October 1995.

[6] Cohen, S., Tyrrell, D.A. and Smith, A.P. Psychological stress and susceptibility to the common cold. *N England J Med* 1991; 325:606-612.

[7] Relyea, R.A. Synergistic impacts of malathion and predatory stress on six species of North American tadpoles. *Environ Toxicol Chem* 2004; 23:1080-1084.

[8] Hauschild, V.D. and Lee, A.P. Assessing chemical exposures during military deployments. *Mil Med* 2004; 169:142-146.

[9] Lam, D.M. and Grubbs, F. The health hazard assessment program. *Army Research, Development, & Acquisition*, September/October 1987; 5-8.

[10] Murnyak, G.R., Leggieri, M.J. and Roberts, W.C. The risk assessment process used in the army's health hazard assessment program – tutorial. *Acquisition Review Quarterly* 2003; 10:200-216.

[11] Stuhmiller, J.H., Long, D.W. and Stuhmiller, L.M. An internal dose model of incapacitation and lethality risk from inhalation of fire gases. *Inhalation Toxicology* 2006; 18:347-364.

[12] Chan, P.C., Ho, K.H., Kan, K.K., Stuhmiller, J.H. and Mayorga, M.A. Evaluation of impulse noise criteria using human volunteer data. *Journal of Acoustical Society of America* 2001; 110:1967-1975.

[13] Stuhmiller, J.H., Ho, K.H., Vander Vorst, M.J., Dodd, K.T., Fitzpatrick, T. and Mayorga, M. A model of blast overpressure injury to the lung. *Journal of Biomechanics* 1996; 29:227-234.

[14] Vasterling, J.J., Proctor, S.P., Amoroso, P., Kane, R., Heeren, T. and White, R.F. Neuropsychological outcomes of Army personnel following deployment to the Iraq War. *JAMA* 2006; 296:519-529.

[15] Rona, R.J., Hooper, R., Jones, M., Hull, L., Browne, T., Horn, O., Murphy, D., Hotopf, M. and Wessely, S. Mental health screening in armed forces before the Iraq war and prevention of subsequent psychological morbidity: follow-up study. *British Medical Journal* 2006; 333:991-995.

[16] Chesbrough, K.B., Ryan, M.A.K., Amoroso, P., Boyko, E.J., Gackstetter, G.D., Hooper, T.I., Riddle, J.R. and Gray, G.C. The Millenium Cohort Study: a 21-year prospective cohort study of 140,000 military personnel. *Mil Med* 2002; 167:483-488.

[17] Smith, T.C., Smith, B., Corbeil, T.E., Riddle, J.R. and Ryan, M.A.K. Self-reported mental health among US military personnel prior and subsequent to the terrorist attacks of September 11, 2001. *J Occup Environ Med* 2004; 46:775-782.

[18] http://biodefense.georgetown.edu/projects/argus.aspx (Last accessed 8 February 2007).

[19] Michalek, J.E. Pharmacokinetics of TCDD in veterans of Operation Ranch Hand: 10-year follow-up. *J Toxicol Environ Health* A 1996; 47:209-220.

[20] Michalek, J.E. Postservice mortality of Air Force veterans occupationally exposed to herbicides during the Vietnam War: 20-year follow-up results. *Mil Med* 2005; 170:406-413.

[21] Abbott, R.D., Ross, G.W., White, L.R., Sanderson, W.T., Burchfiel, C.M., Kashon, M., Sharp, D.S., Masaki, K.H., Curb, J.D. and Pertorvitch, H. Environmental, life-style, and physical precursors of clinical Parkinson's disease: recent findings from the Honolulu-Asia aging study. *J Neurol* 2003; 250:30-39.

[22] Weisskopf, M.G., O'Reilly, E.J., McCullough, M.L., Calle, E.E., Thun, M.J., Cudkowicz, M. and Ascherio, A. Prospective study of military service and mortality from ALS. *Neurology* 2005; 64:32-37.

[23] Page, W.F. Long-term health effects of exposure to sarin and other anticholinesterase chemical warfare agents. *Mil Med* 2003; 168(3):239-45.

[24] NATO. Study Draft 2 AMEDP-21, "Deployment Health Surveillance", Brussels, Belgium; NATO Standardisation Agency, September 2006; 2-2.

Chapter 11 – HEALTH RISK COMMUNICATION: INTENDED AND UNINTENDED CONSEQUENCES OF BIOMONITORING

by

B. Liljedahl[1], C. Ivgren[2], and K.E. Friedl[3]

[1]Division of NBC Defence, Swedish Defence Research Agency (FOI-skydd), Umea, Sweden;
[2]Swedish Defence Forces, Stockholm, Sweden
[3]U.S. Army Medical Research & Materiel Command, Fort Detrick, Maryland, U.S.A.

11.1 ABSTRACT

The introduction of biomonitoring for assessment of troops' exposure to toxic hazards in operational environments will provide new opportunities to gain important answers and data on health hazards. At the same time, such monitoring may raise new concerns and trigger a need for communicating to troops why, how and for whose benefit the monitoring is conducted; properly communicated, explanations about biomonitoring may increase confidence that preventive medical measures are being taken and that there is genuine concern about protecting soldier health. The perception of risk and behavioural response is affected by the manner in which soldiers receive their information about biomonitoring efforts. This chapter attempts to stress, through a very brief overview, why some key factors such as risk perception, social amplification and risk communication, will be important issues to address, if a future use of biomonitoring is to be successfully implemented in operations, and how biomonitoring could, in turn, benefit from being made more easily understood and familiar to troops, relatives and the media. The chapter briefly discusses such concepts as the psychometric paradigm of risk perception and social amplification of risk, from an operational point of view, and what this may mean in the context of soldiers' perception for true or perceived toxic exposure. It comments on how soldiers' risk perception (and need for risk communication) may differ not only between troops and on an individual basis, but also due to other stress factors within the mission or home conditions, during and after a mission. Finally the chapter discusses how and why risk communication is essential when developing new techniques of biomonitoring for toxic hazards in operational environments.

11.2 SCOPE OF HEALTH RISK COMMUNICATION IN BIOMONITORING

Health risk communication, and health risk communication in military operational environments, is a science in itself, with worldwide growing knowledge on an academic as well as everyday operational level. Operational exposures issues are currently addressed, and new important understanding of troops' reactions to health topics gained [1 – 3].

This chapter makes no attempt to address the full complexity of the field. Our goal is to point out the need for the early introduction of risk communication aspects, into the fast development of new biotechnologies for the assessment of toxic hazards in operational environments.

Biotechnology, from a risk perception perspective, has been identified as one of the new technologies in which non-technical people today perceive many risks that may seem irrational to "experts." Additionally, the divergence in understanding and association of the meanings of words such as "exposure", "risk", "hazard" and "toxic" among lay people and experts [4] is of interest. Sampling and assessment for exposures have been applied during operations for a long time, resulting in routines designed to deliver not only

chemical data analysis, but to answer concerns among the soldiers such as; "…if they are monitoring, there has to be a hazard" or "…why are you only sampling in other camps, not in our units?" or "…now I feel sick - why can't I see the results?" In all, it's well known that monitoring and sampling in operations without efficient risk communication is likely to pose an additional burden and may introduce more stress in troops. Therefore, we must ask what particular challenges await, when introducing biomonitoring of troops in war, peace enforcing, or peacekeeping missions?

11.3 KEY RISK ISSUES, SOME EXAMPLES

How will a soldier operating in war or on peacekeeping missions perceive toxic hazards in theatre to which he or she might be exposed? The question is appropriate when discussing new biotechnologies, intending to provide a scientific answer to questions on toxic hazards and exposures among troops. In alerting soldiers to potential health risks through direct information (or indirectly signaling potential risks through monitoring), it is important to present accurate information about a credible health threat that properly arms the individual against the threat without inadvertently causing counterproductive behaviors such as panic (Figure 11-1). "Ebola virus" may provoke panic in civilian populations. In contrast, properly informed and prepared soldiers should be well able to defend themselves against Ebola virus, without adopting a careless or cavalier attitude towards a deadly threat, but also with confidence that this is a fully manageable disease threat.

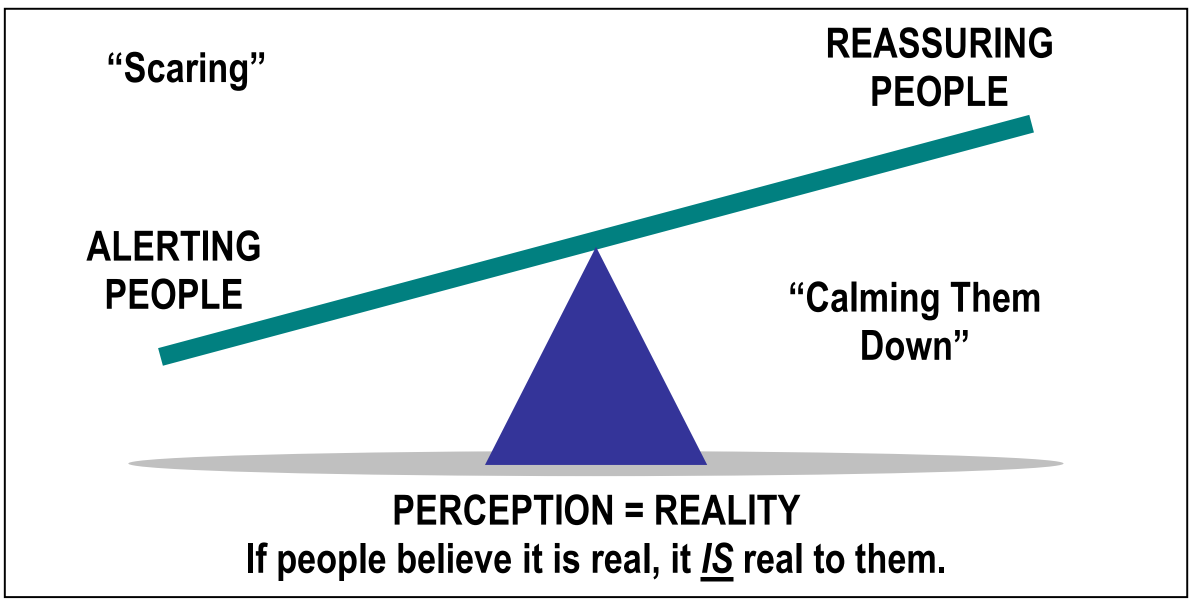

Figure 11-1: There is a Fine Balance in Health Risk Communication Between Providing Accurate Information that Prepares Soldiers Against a Health Risk and Causing Counterproductive Emotional Behaviors. It is important to get this right because the perceived message becomes reality.

Despite a scientific approach, where the risk can be calculated as a mathematical outcome of likelihood and consequences, there may be a difference between the predictable risk and the perception of risk – perceptions, not science, will take precedence in the behavioural outcomes. Current authorities in the field of Force Health Protection maintain that "*there is no such thing as an "objective risk*" [5] and that "*perception equals reality"*[6]. Thus, not only physical exposure, but anxiety about a perceived exposure, and the costs for military forces or society to address them, are highlighted in some recent deployment health issues including

Gulf War Syndrome, post traumatic stress disorders (PTSD), depleted uranium (DU) or Severe Acute Respiratory Syndrome (SARS).

Is this respect a few comments can be made on:

a) The psychometric paradigm (key factors in risk perception);

b) Social amplification of risk; and

c) Health risk communication.

11.3.1 The Psychometric Paradigm – Operational Aspects

11.3.1.1 Key Factors in the Psychometric Paradigm

Why do deployment operations attract more media coverage due to rumours of troops' potential or perceived exposure to a toxic substance, than does reporting the lethal outcome of traffic accidents – one of the highest non-battle causes of death in world wide operations? What risks are troops willing to take, as a part of their mission? And how do these questions interact with those surrounding biomonitoring? Risk means different things to different people, and wide variances are typical between "experts" and the lay public (or soldiers). The psychometric paradigm [7] identifies some significant key factors of concern in risk perception:

- Perceived controllability;
- Catastrophic potential;
- Dread;
- Unfamiliarity (strange odour, invisible, etc.);
- Perceived voluntariness of exposure;
- Who is affected?; and
- "Tampering with nature".

Biomonitoring in operations will address hazards that in many cases are unfamiliar to troops, and it can be noted that several of the key factors for risk perception are in the mainstream when addressing exposures of toxic hazards in operational environments, as well as new biotechnologies for the assessment of such exposures. From an exposure perspective, components such as "unfamiliarity" and "tampering with nature" are part of a spectrum that has recently been described in "The spectrum of threats" by NATO/EAPC in the new draft policy on Environmental and Industrial Health Hazards (EIHH)[8], as depicted in Figure 11-2.

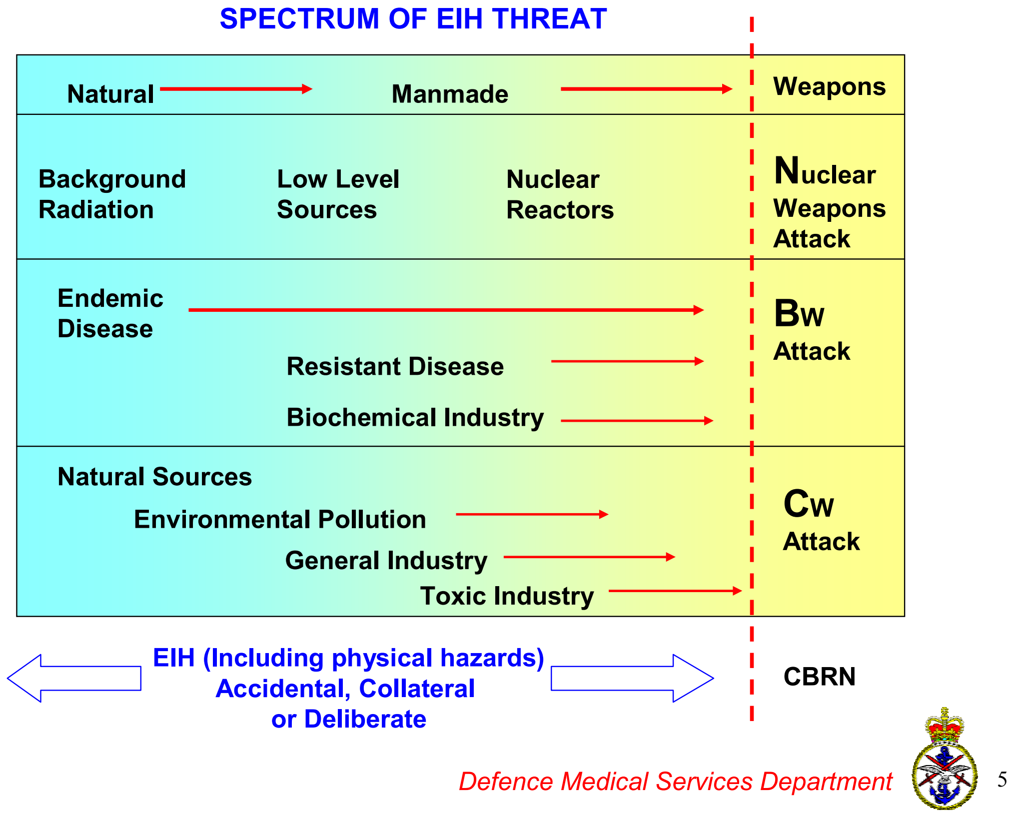

Figure 11-2: Spectrum of Environmental and Industrial Health Threats.

11.3.1.2 Motivation – One Brick in the Foundation of Risk Perception and Exposure Resistance

The psychometric paradigm also highlights the issue of perceived voluntariness of exposure [9]. In this context it is interesting to address the reported data on Motivation and Self-Image from deploying troops. How voluntary do they regard their participation to the mission and its potential hazards? Several studies have sought the key motivation for deploying to international operations among drafted soldiers and conscripts. One study of German troops [10] reported that 64% of troops regard themselves in the category "helper in Uniform," 22% as "leader and Education," 6% as "Careerist," 5% as "Martial Adventurer," and 3% as "Male Warrior." The main motivating factors among 1238 peacekeepers in Swedish UN battalions in BiH were identified to be military challenge, private financial reasons, sensation seeking or humanitarian and altruistic will [11]. Motivation among Austrian conscripts serving on the Golan Heights and in BiH 1996 – 97 [12] and among Norwegian peacekeepers serving in Lebanon (1978 – 1991) [13] was identified to be mainly associated with "high pay" and the chance to experience "adventures".

11.3.1.3 Trust in Commander and Experts – Who do Soldiers Listen to?

Research indicates that social trust of those who manage a hazard is strongly correlated to judgments about the hazard's risk and benefits [14]. Furthermore it is suggested that the lay public relies on social trust when making judgments of risks and benefits, when personal knowledge about a hazard is lacking. Troops are likely to have personal experience regarding operational DNBI (disease and non battle injurious) hazards such as traffic accidents, smoking hazards, malaria, heat stress and injuries resulting from physical training, whereas exposures to toxic hazards may be regarded as more unfamiliar – thus calling for a need to rely on social,

rather than personal, risk assessments. For this reason, trust in the chain of command, and commanders' preparedness to be able to support troops in a trustworthy manner, on matters of exposures to toxic hazards and potential biomonitoring is of special concern. Soldiers may become complacent in protection against hazards that are generally familiar, while unfamiliar hazards carry a high risk for suspicion and over-reaction. These extremes have to be countered in effective health risk communication.

The importance of a favourable relationship with peers and commanders for an increased likelihood of a satisfactory overall evaluation of the service period has been identified in several studies on soldiers in combat situations [15 – 17] as well as peacekeeping missions [18]. This will not only be important in soldiers' potential for social amplification of risk perception in general, but will also lay an important foundation when addressing the issue of toxic hazards and potential biomonitoring on operations.

In practice, experiences from theatre have showed that troops will rely more on the message passed by well-informed and trusted commanders rather than by experts [19, 20]. On the other hand, if a commander has not gained the trust of his/her troops, suspicion of biases, contradicting agendas and lack of concern will hamper risk communication, and experts may be regarded as more neutral actors. It may be important, from a biomonitoring view, that soldiers' view of risks in general and "acceptance" of exposures to toxic hazards, may differ severely depending of motivation and trust in the chain of command. Therefore, introduction and conduct of biomonitoring studies must be made with an understanding that unmotivated troops may be more sensitive with respect to the need for risk communication.

Experts also have responsibility for conveying health risk information to commanders with appropriate confidence levels, caveats, and a balanced perspective on the state of the knowledge; commanders make operational decisions that will be influenced by this information, but only if they also have confidence in their experts. In fact, scientific predictions can be wrong since these are only "best available" solutions to problems with real world complexities, usually extrapolated from highly controlled laboratory experiments or previous experience (e.g. epidemiological associations). Wrong predictions delivered with a high degree of certainty obviously contribute to health risk communication problems with the rapid erosion of credibility.

11.3.2 Social Amplification of Risk

11.3.2.1 Introduction

Events pertaining to hazards interact with psychological, social, institutional and cultural processes in a way that can amplify or attenuate perceptions of risk and shape risk behaviour [21]. The media plays a significant institutional role in amplifying or attenuating risk perception through decisions on what to report and how. Other sources of information such as the internet can have enormous significance and include unique opportunities for deliberate misinformation by an enemy. Troops are subjected to different types of stress during the mission cycle, also including post mission life. Mission-related toxic hazards and exposure or perceived exposure to such hazards may play an important role in the process of social amplification and actually promote stress. Furthermore, experience shows that perception of toxic exposure may spread very rapidly among troops, resulting in every-day expressions as "chemical epidemic" in theatre [22]. Biomonitoring, with its potential to provide a broad spectrum of chemical data analysis, may be looked upon as a potential "answer" to problems it was never intended to address.

11.3.2.2 Toxic Hazards, Stress and Social Amplification of Risks

As discussed in Chapters 9 and 10, an exposure situation (whether physical or perceived), should be placed into its mission lifetime context, and into the overall stress load. Tasks and situations of danger and/or combat

may differ significantly between different missions as well as during the mission cycle. Disregarding obvious individual differences, it is not unusual for soldiers in peacekeeping missions to describe less dangerous periods as "less positive"[23]. Furthermore, it has been discussed that it does not always seem to be the exposure to life-threatening events in itself that is the main stressor, but the conflict between strong aggressive impulses seeking an outlet and the inability to express them [24]. In other words, operational stressors overlaying toxic threats may include both external challenges and internal psychological conflicts. These latter stressors have become more significant in new peacekeeping roles for soldiers where they may be limited in their permissible responses.

Operational experiences during the past decade have shown that exposure, or perceived exposures, to toxic hazards may often serve as a "carrier" or amplifier for soldiers' frustration and stress levels [22]. During mission phases when activity rate is low, health concerns are likely to be raised [25], and concerns about potential health hazards linked to sometimes less expected issues such as bottled water, high-voltage lines, and distant ambient pollution (visible, but not necessarily with exposure routes that might affect troops) become a point of discussion [26].

The importance of a stable social network at home, as well as mutual respect with the chain of command, for the wellbeing of the soldier has been addressed in numerous studies. In all, there seem to be minor or no differences among soldiers from a professional military force, or a force of volunteers, regarding a positive outcome from a strong family support, good relations with commanders and peers (unit cohesion), and a certain degree of exposure to stressful events. This key factor for the soldiers' wellbeing may be related to post-mission stress reactions and, if a person perceives that the mission has directly or indirectly given rise to family problems or breakups, the individual may associate stress reactions with incidents (or exposures) that took place during the mission, rather than being properly attributed to a stressful private situation [27]. The similarity between diffuse symptoms of individual stress and some toxic exposures may add to a complex picture. For this reason studies evaluating potential chemical or other exposure effects today try to include the individual social situation, and great efforts are being made to include this factor in different deployment and post deployment questionnaires. The importance of family relationships and support in soldier health and performance is better recognized today than in the past, and it has gained greater importance for some armies today which have increasing proportions of married soldiers. Family and unit cohesion can, in turn, be affected by individual soldier deployment exposures. The importance of toxic chemical exposures, traumatic stress conditions, and other injurious exposures such as mild traumatic brain injury (mTBI) to changes in a soldier's mood (e.g. rages, depression) and behaviour is now beginning to be appreciated [28 – 30]. The potential interactions of these three components, each of which are important in current deployments, is completely unknown – but there is increasing public awareness of these threats and a growing need to clearly explain the known and suspected health consequences.

From this perspective, it may be discussed if and how individuals or groups that have been subjected to biomonitoring (or excluded from such a monitoring) and later perceive severe post deployment stress reactions, or even PTSD, will have an interest in results and an analytical outcome from the bio sampling. This highlights the need for preparation of honest risk communication addressing, among others, the four criteria proposed by the WHO before considering screening a population:

- Only screen for diseases that are common and/or may result in great suffering for the individual;
- Only screen when diagnostic methods are sensitive enough to identify a disease but specific enough to exclude the disease among healthy or exclude false positives;
- Only screen when there is an efficient treatment that can reduce the suffering; and

- Finally, there is a need for a cost-benefit analysis, not only financial, but including potential physical and/or psychological unease due to the screening procedure [31].

Intermediate screening approaches that detect changes in markers of effect that are not necessarily strong predictors of a specific disease outcome but which indicate a change in response to deployment are problematic and may, in themselves, compromise wellness. Thresholds of action for new measurable biomarkers may not be readily determined after the fact, when health monitoring may be confused with validation of an association between biomarker and outcome (e.g. detectable urine uranium after depleted uranium exposure). Necessary epidemiological research must be carefully explained to commanders and soldiers so that everyone has realistic expectations in advance of the reporting of results.

11.3.2.3 Media

The media coverage of the development of events in a mission area tends to be important to the deployed soldiers as well as to the relatives at home [32]. While news coverage is normally high during the early deployment phases, it tends to diminish during a long term ongoing mission. This may change rapidly, in the event of an exposure or perceived exposure situation in the mission, such as a focus on depleted uranium, rumours of “faecal dust” (Afghanistan) [33], and fear of lead exposure Mitrovica, Kosovo [34]. Returning to the “Spectrum of Threat” (Figure 11-2) it may be noted that media coverage tends to increase the further the exposure is perceived to be to the right of the figure (i.e. towards manmade warfare agents). A decision to introduce biomonitoring among troops, or some of them, is not unlikely to become a news event of interest for the media, raising questions about a presumed, maybe unknown, health risk for the soldiers. Following the first Gulf War experience, the public focus has been in the opposite direction for the U.S., with questions raised about why there is not more biomonitoring and health surveillance of deployed soldiers, with an unrealistic expectation that everything about a soldier’s health status will be explained by measurements conducted using serum samples taken before and after a deployment.

Media reports clearly affect the public perceptions of reality. Every time a new national announcement occurred concerning Gulf War Illnesses in the U.S., there was a surge in new enrollments to the DoD Gulf War health registry from service members concerned about their health (Figure 11-3). The most notable spikes followed news about neurotoxic chemical threats, including discussion about DEET and low-level sarin exposures and the hypothesized associated health risks.

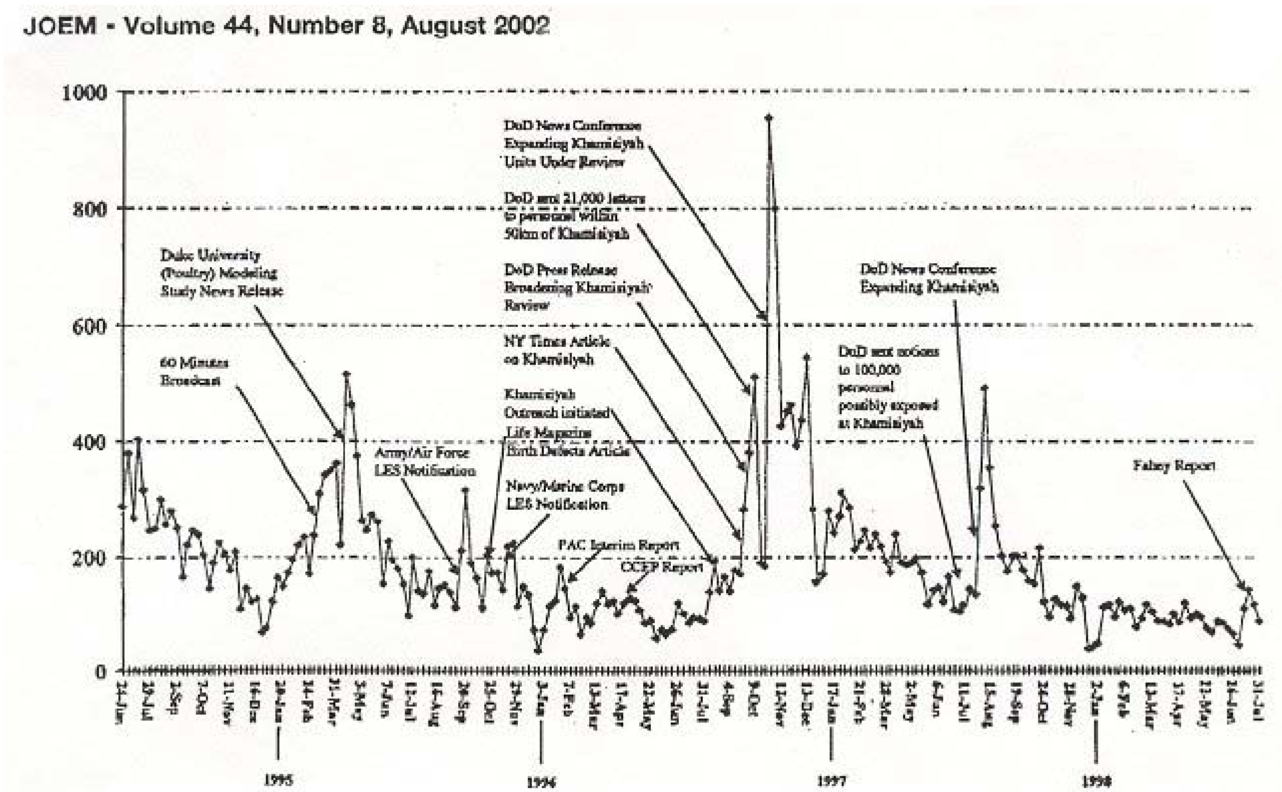

Figure 11-3: New Enrollments to the Gulf War Health Registry in Relationship to DoD News Releases and Reports between June 1994 and July 1998 (from [35]).

11.4 HEALTH RISK COMMUNICATION AND BIOMONITORING

The fast developing technique of biomonitoring and biomarkers has provided a new perspective on exposure assessments. Recent biomonitoring studies have examined the levels of 200 chemicals. However, biomonitoring data, by themselves, are not very useful in helping consumers (soldiers or relatives) to understand their individual health risk [36]. A major challenge facing those who conduct biomonitoring programs is how to best communicate the information to the troops.

Will troops trust the health risk communication provided? In this context, there are well-known factors of certain concern when discussing the need for communication on exposure and biomonitoring with deploying troops. Risk judgments need to address [37] the concern among troops that "experts" may be biased, "experts" may be motivated by self-serving interests and values, "expert" risk judgments are not absolute and that "lay" risk judgments should not be discounted. In the end, what risks are deemed "tolerable" or "acceptable" will depend on how those involved view the data upon which the risk measure is based, and whose values are considered. Some aspects are discussed below.

The purpose of biomonitoring and the methods described in this report is to serve as one of many tools in the battle to ensure the health of deploying troops. The benefits, from a medical and scientific point of view are many: an increased possibility to evaluate and compare the health status of a soldier before and after deployment; an increased possibility to measure effects on an individual basis, rather than estimate potential

individual exposure rate from ambient monitoring; and not least, the potential for gathering objective data in numeric form which could facilitate statistical evaluations that will improve the scientific knowledge on true exposure rates during deployments, thus creating a sound base for future preventive medicine activities.

At the same time, the complexity of health status, stress factors, neurobehavioral effects and exposure rates discussed in this report is well known. The evaluation and transforming of biomonitoring data into assessed individual health impacts, must thus be set into its true context, if the goal is to address exposure incidents during deployments. Deployed personnel can be expected to respond differently to an exposure incident, depending not only on genetic and physiological sensibility, but also on mental factors such as expectations, motivations, trust in command, and relation to mission task.

Furthermore, toxic exposure monitoring in general, and biomonitoring in particular, may induce stress in troops well as in relatives at home, if not conducted with rigorous risk communication. In response to questions raised about post deployment illnesses after the first Gulf War in 1991, the U.S. DoD invested substantial resources into a thorough series of investigations, reviews, and information exchanges, providing a comprehensive health risk communication program that has become a model for how deployment toxicology issues should be addressed [38].

Key factors for success in communicating exposure hazards with troops, are well known [25], and biomonitoring for exposure among deploying troops will highlight key factors such as the need for:

- Trust in command;
- Trust in experts;
- Belief in the mission;
- Long-term follow up on risk communication after mission;
- Information *sharing* – not top down!;
- Trust in new techniques and, above all;
- The realisation that *a perceived risk will become a true risk.*

Information should be shared with respect to the fact that troops are likely to find the new technology of biomonitoring, and the toxic hazards it's intended to address, unfamiliar, and thus look for sound, practical and robust ways:

- To reduce fears of:
  - The unknown,
  - Spectacular rather than common death, and
  - Invisible threats and threats rather than those associated with strange odours;
- To encourage the disappearance between lay people and experts in their perception of the meaning of words such as; "toxic", "risk" " hazard", etc.; and
- To recognise the importance of toxic hazards as "carriers" for stress factors.

With early risk communication and information sharing, amongst not only troops, but with the chain of command, relatives and not least the media, some of the challenges for biomonitoring as a tool in preventive health may be overcome.

The chapter has been written with acknowledgement to LtCol Ken Roberts, Army Military Directorate, UK, Dr Lori Geckle USACHPPM, USA, and Anne Kari Rom, Senior Advisor, Norwegian Army.

11.5 REFERENCES

[1] http://chppm-www.apgea.army.mil/risk/resources.aspx (Last accessed 9 February 2007).

[2] http://www.forces.gc.ca/health/information/engraph/op_health_home_e.asp (Last accessed 9 February 2007).

[3] http://www.ukresilience.info/preparedness/risk/index.shtm (Last accessed 9 February 2007).

[4] MacGregor, D.G., Slovic, P. and Malmfors, T. "How exposed is exposed enough? Lay inferences about chemical exposure." Risk Anal, 1999; 19(4):649-659.

[5] Roberts, K. "Risk perception and risk communication", drawing on Slovic "Perception of risk". Science 1987; 236:280-285.

[6] Postlewaite, C. US Department of Defense Deployment Health Surveillance Program. Presented at First Environmental and Industrial Health Hazard (EIHH) workshop, Umeå Sweden 2004. Available online at: www.eihh.foi.se (Last accessed 9 February 2007).

[7] Renn, O. 'Risk perception and risk management; A review, part 1: Risk perception'. Risk Abstract 1990; 7:1-9.

[8] NATO/Euro-Atlantic Partnership Council. "Policy on Environmental and Industrial Health Hazards", 2006, currently under revision by member states.

[9] Starr, C. 'Social Benefit versus Technological Risk'. Science 1969; 165:1232-1238.

[10] Tomforde, M. "Motivation and self-Image among German peace keepers". International Peacekeeping 2005 Winter; 12(4):576-585.

[11] Johansson, E. and Larsson, G. "Swedish peacekeepers in Bosnia and Herzegovina: A quantitative analysis". International Peacekeeping 2001 Spring; 8(1):64-76.

[12] Kernic, F. and Haas, H. "Warriors For Peace". Frankfurt am Main: Peter Lang, 1999; 83.

[13] Forsvarets Sanitet, UNIFIL-Undersökelse. Resultat og anbefalinger, Oslo, Norway: Mil/Huseby, 1993.

[14] Siegrist, M. and Cvetkovich, G. Perception of hazards: The role of social trust and knowledge. Risk Anal 2000; 20(5):713-719.

[15] Belenky, G. (ed.), Contemporary Studies in Combat Psychiatry, Westport, CN; Greenwood Press, 1987.

[16] Gal, R. Unit morale: from a theoretical puzzle to an empirical illustration – An Israeli example. J Appl Soc Psychol 1986; 16(6):549-64.

[17] Gal, R. and Jones, F.D. A psychological model of combat stress. In: Jones FD et al. *War Psychiatry*, Washington DC: TMM Publications at the Walter Reed Army Medical Center, 1995; 133-48.

[18] Lamerson, C.D. Peacekeeping stress: Testing a model of organizational and personal outcomes. (Doctoral dissertation, University of Guelph, Ontario), Dissertation Abstracts International, 1996; 57, 4070B.

[19] Roberts, K. UK experiences on communicating risks during a range of UK military operations 1996-2006. 2nd International Environmental and Industrial Health Hazards Workshop. Rijswijk, the Netherlands, 5-6 March 2006.

[20] Major, C. Ivgren Swedish Armed forces experiences on communicating exposure risks during KFOR mission, Kosovo 1999-2006. 2nd International Environmental and Industrial Health Hazards Workshop, Rijswijk, the Netherlands, 5-6 March 2006.

[21] Kasperson, R. et al. The social amplification of risk: A conceptual framework. Risk Anal 1988; 8(2):177.

[22] Rom, A.K., Senior Adviser, Norwegian Army, TRADOK, Oslo, personal communication.

[23] Vogelaar, A.L.W., Soeters, J.L. and Born, H. "Working and living in Bosnia: experiences of Dutch IFOR soldiers", in Netherlands Annual Review of Military studies: the Bosnian Experience. Tilburg, The Netherlands: Gianotten BV, 1997; 126.

[24] Johansson, E. and Larsson, G. Swedish peacekeepers in Bosnia and Herzegovina: A quantitative analysis. International Peacekeeping, 2001 Spring; 8(1):64-76.

[25] Roberts, K. Health-risk management – a developing methodology for military operational support. Presented at: 2nd Workshop on Environmental and Industrial health hazards (EIHH), Rijswijk, the Netherlands, 5-6 March 2006.

[26] Ivgren, C. Experiences from deployments within e.g. KFOR, ISAF and MONUC. 2nd International Environmental and Industrial Health Hazards Workshop, Rijswijk, the Netherlands, 5-6 March 2006.

[27] Bache, M. and Hommelgard, B. Danske FN-soldater: Oplevelser og stressreaktioner (Danish UN soldiers, Experiences and stress reactions) Köbenhavn, DK: Forsvarets Center for lederskab, 1994.

[28] Labbate, L.A. and Warden, D.L. Common psychiatric syndromes and pharmacologic treatments of traumatic brain injury. Curr Psychiatry Rep 2000; 2:268-273.

[29] White, R.F. and Proctor, S.P. Solvents and neurotoxicity. Lancet 1997; 349:1239-1243.

[30] Bleich, A., Koslowsky, M., Dolev, A. and Lerer, B. Post-traumatic stress disorder and depression. An analysis of comorbidity. Br J Psychiatry 1997; 170:479-482.

[31] Wilson, J.M.G. and Junger, G. Principles and Practices of Screening for Diseases, World Health Organization, Geneva, Switzerland; WHO, 1968.

[32] Johansson, E. and Larsson, G. A model for understanding stress and daily experiences among soldiers in peacekeeping operations. International Peacekeeping 1998 Autumn; 5(3):124-41.

[33] Canadian experiences on the event of " faecal dust". Available online at: http://www.forces.gc.ca/health/information/engraph/op_health/engraph/MapleLeaf_ReducingHealth_home_e.asp

[34] Swedish, UK and Norwegian experiences within KFOR of perceived lead exposure, Mitrovica Kosovo. 2nd International Environmental and Industrial Health Hazards Workshop, Rijswijk, the Netherlands, 5-6 March 2006.

[35] Smith, T.C., Smith, B., Ryan, M.A.K., Gray, G.C., Hooper, T.I., Heller, J.M., Dalager, N.A., Kang, H.K. and Gackstetter, G.D. Ten years and 100,000 participants later: occupational and other factors influencing participation in U.S. Gulf War health registries. J Occup Environ Med 2002; 44:763.

[36] Paustenbach, D. and Galbraith, D. Biomonitoring and biomarkers; Exposure assessment will never be the same. Available online at: ehp online – http://dx.doi.org/ ehp8755 doi: 10.1289 (Last accessed 26 April 2006).

[37] Fischoff, Slovic, Lichtenstein. Facts and fears: Understanding perceived risk. In: Societal Risk Assessment. Schwing R, Albers W (Eds). New York NY; Plenum Press, 1990.

[38] http://www.gulflink.osd.mil/library/osagwi_reports.html (Last accessed 9 February 2007).

Chapter 12 – RECOMMENDATIONS

12.1 THE PANEL

Recommends:

- That increased attention be paid to the monitoring of pulmonary function and association of long term health risks associated with particulate exposures (e.g. "Kabul cough", use of oxidative stress markers; and leukotrienes in breath condensate);
- More international cooperation on key issues such as the safety of permethrin in uniforms;
- The development of comprehensive NATO/PfP database(s) on biomarkers and monitoring technologies, to assist in improved information sharing and standardization of methods (e.g. perhaps as an allied medical publication);
- Notice be taken of the fact that while biomarkers of exposure such as protein adducts of specific compounds can be highly effective approaches to monitoring toxic chemical exposure risks, biomarkers of effect are a problem – e.g. cholinesterase testing has no consensus on method or actionable thresholds; oxidative stress markers are not particularly useful in guiding medical decisions or predicting any health risks;
- More research to resolve the facts that acute and chronic effects of militarily-relevant solvent exposures are still poorly defined and good biomarkers of effect (behavioural, biochemical, and neurological) have not been determined; and
- That the need be recognised for translational research on the effectiveness of monitoring and interventions for the military health monitoring and surveillance tools.

Annex A – NATO/PfP WORKSHOP ON ENVIRONMENTAL AND INDUSTRIAL HEALTH HAZARDS AND PUBLIC HEALTH CONCERNS IN INTERNATIONAL MISSIONS

14 – 15 October 2004, Umea, Sweden

A.1 MEETING PRESENTATIONS

NATO's Updated Nuclear, Biological and Chemical Agent Threat Evaluation
LTC Cornelis Wolterbeek, NATO WMD Centre

Lessons from the DoD Gulf War Illnesses Research Investment – Neuroepidemiology, Environmental Exposures, and Soldier Well-being
Col Karl E. Friedl, Ph.D., U.S. Army Research Institute of Environmental Medicine, Natick, MA

UNEP Post-Conflict Activities
Pekka Haavisto, Chairman, UNEP Post-Conflict Assessment Unit

Deployed Environmental Health Risk Management in the Canadian Forces
Chris Knowlton, EIHH/PHC Project Director, National Defence Headquarters, Canada Bulgarian

Experience in Medical Support of Bulgarian Military in Peacekeeping Missions
Julian Raynov, MD, PhD, Head of Department, Military Medical Academy, Bulgaria

Inventory of Chemicals and Risk Assessment at Metal Factory in Kosovo
Rauno Pääkkönen, Finnish Institute of Occupational Health

Lessons learned from Kosovo concerning hazardous chemicals – example of hydrocyanic acid in Mitrovica
Yves Kovalevsky, French Army NBC-Defence Center

The Qarmat Ali Water Treatment Plant, Southern Iraq: UK EIH Policies and Procedures in Action
Maj Ken Roberts, Defence Medical Services Department, UK

Swedish Experience from Africa (Democratic Republic of Congo and Liberia)
Maj Claes Ivgren, Joint Forces Command J4, Swedish Armed Forces

Malaria outbreaks during the operation Unicorn (Ivory coast). Lessons learned to improve forces protection
Dr. Remy Michel, Inst de Médecine Tropical du Service de Santé des Armées, Marseille, France

Operational health and safety requirements and identifying short term and long term research and development to meet their needs
Maj Yvonne Severs, CD, MSc, DRDC Toronto, Canada

Risk Assessment in Finnish Peacekeeping Missions
Kyösti Lehtomäki, Cpn, Finnish Defence Forces

Swedish Experience from Kosovo in Mapping Risks Other Than Attack (ROTA) and Associated problems *Anders Grönlund, former Head of GIS Cell, KFOR, Kosovo*
US Department of Defense Deployment Health Surveillance Program *Dr. Craig Postlewaite, Department of Defence/Health Affairs*
Predeployment Environmental Risk Assessment *Maj Herman Steenbergen, Dutch Army Forces, The Netherlands*
GIS and Internet as resources for distributing know-how to Swedish missions *Christina Edlund, FOI NBC-Defence, Sweden*

These presentations from the 1st EIHH can be viewed and downloaded from www.eihh.foi.se

A.2 CONCLUSIONS

A.2.1 Successful Meeting

- It was generally agreed that the Umea NATO/PfP Workshop had been successful and that it was worthwhile to continue with meetings dealing with health-related issues of troops on operational deployments.
- The Netherlands promised to investigate hosting the meeting next year.
- The follow-on meeting should focus on particular themes instead of general discussions of EIHH and PHC-related problems.
- Sweden volunteered to keep the workshop website open and updated until the next meeting is scheduled. All relevant EIHH and PHC information that participating nations can provide to populate the website is welcome (http://www.eihh.foi.se).

A.2.2 Information Sharing

- The sharing of open source information in the form of websites, website links, databases, books, and other material might be a useful starting point for wider collaboration. This would cover all background data useful for information collection regarding EIHH/PHC issues in different parts of the world. Sweden volunteered to collect this information and make it available to the workshop participants.
- Official national information, not subject to severe security restrictions, but for example, classified "for official use only," cannot be made available unrestricted. It was suggested that such information could be compiled anyway and protected through limiting access. Nations could receive access on request or through a password.
- Compilation of open source and official information could become a deliverable from HFM-057/RTG-009.
- It was made clear by the NATO WMD Centre representative that NATO is organising databases for information sharing among NATO and Partner nations. Therefore, NATO would be willing to assist in organising databases for EIHH and PHC topics.

A.2.3 Governing Body

- It was recommended that a body to champion EIHH and PHC-related issues be found. Whether that body should more appropriately belong to the CBRN or medical community was a point of discussion.

- One way forward would be to write a consideration paper for inclusion of EIHH and PHC-related issues in a suitable task or working group to the DGP (Senior Defence Group on Proliferation; reports to the North Atlantic Council) for policy guidance.

- The questions are: 1) whether EIHH/PHC issues lie within the domain of CBRN force protection, 2) the responsibilities and involvement of DGP, and 3) whether this lies within the domain of he health care community. In the case of the latter, the alternative would be to request guidance from COMEDS (the Committee of the Chiefs of the Military Medical Services in NATO; COMEDS is the senior military medical body of the Alliance, and reports directly to the Military Committee). For operational-level work it would be preferable to task an existing working group, rather than establish a new one for this purpose, and LG-7 (Landgroup 7, which reports to the NATO Army Armaments Group) should be involved.

- As many of the participants of the workshop had met in HFM-057/RTG-009, this group could carry the EIHH and PHC torch while the possibility of a permanent home is under investigation. Given that this debate is largely a medical or CBRN issue, it is clear that regardless of who takes the lead, considerable liaison will need to be maintained between the two communities.

Annex B – 2ND INTERNATIONAL WORKSHOP ON ENVIRONMENTAL AND INDUSTRIAL HEALTH HAZARDS AND PUBLIC HEALTH CONCERNS IN INTERNATIONAL MISSIONS

5 – 6 April 2006, Rijswijk, Netherlands

B.1 MEETING AGENDA

April 5

09:00 – 09:15	Brigade-General Van der Meer (RNLA): Welcome
09:15 – 10:00	Dr. Paul Knechtges (USACEHR): keynote lecture: 'New biotechnologies for environmental health monitoring'
10:00 – 10:15	Coffee
10:15 – 10:35	Dr. Daan Noort (TNO Defence, Security and Safety): 'Persistent biomarkers of exposure'
10:35 – 11:00	Maj. Klaus Braun (Danish Army): 'Accidental poisoning with organophosphates of workers working in a garbage dump'
11:00 – 11:30	Mrs. Birgitta Liljedahl (FOI): 'Deployment experiences, vision on hazard management'
11:30 – 12:00	Maj. Roger Tremblay (Canadian Forces): 'Canadian Forces Deployable Health Hazard Assessment Team concept'
12:00 – 13:30	Lunch and demonstrations
13:30 – 14:10	Mr. Ted Whiteside (WMDC): 'A Perspective on the Development of the EIH Concept'

April 6

09:00 – 09:15	Dr. Maarten Huikeshoven (MOD-NL): Welcome
09:15 – 10:00	Col. Kees IJzerman (RNLA): keynote lecture: 'The risk assessment of occupational and environmental hazards'
10:00 – 10:15	Coffee
10:15 – 10:55	Dr. Gabriele Borla (UNDPKO): 'UN DPKO Environmental programme'
10:55 – 11:25	Col. F. Van Meeteren (RNLA): 'Introduction of RAO in the Netherlands' Armed Forces'
11:25 – 12:00	Mrs. Christina Edlund (FOI): 'Integration and aggregation of unstructured EIH information using web and GIS-solutions / Medical Intelligence knowledge database'
12:00 – 13:30	Lunch and Demonstrations
13:30 – 13:55	Sqn Ldr. Mark Dray (MOD-UK): 'EAPC Policy in management of environmental and industrial hazards on operations'
13:55 – 14:20	Major Kenneth Roberts (MOD-UK): 'Health-risk management – A developing methodology for military operational support'

B.2 CONCLUSIONS

[These conclusions are taken directly from the published summary at www.eihh.foi.se].

A total of 75 participants attended the Workshop from the UN, NATO HQ, a range of NATO Allies and Sweden.

From the presentations and discussions it is apparent that the issue of environmental and industrial health hazards (EIHH) is high on the agenda of all participating nations, especially since most armed forces are active in out-of-area missions. Furthermore, it appears that many nations are struggling with the management of this issue and that there is a need for a standardised approach, especially given the current frequency multi-national deployments, to avoid 'reinventing the wheel'.

A key issue is the ready and timely exchange of information and data on EIHH. This implies that (wherever practicable) such material should not be classified unless absolutely necessary, and should not be confused with Intelligence products. A means of exchanging such unclassified material was established at the 1st EIHH Workshop, when FOI (Sweden) made their website (http://www.eihh.foi.se/) available for this purpose. Since then, only a very few postings have been made, and participants should be encouraged to use this facility more, within the bounds of their national policies on the release of information. Perhaps data and information exchange and availability should be formalised under EAPC arrangements.

The relationships between the Medical and CBRN/NBC Communities for the management of risk across the spectrum of hazard (ranging from naturally occurring hazards, through those arising from the man-made environment to those posed by 'classic' CBRN/NBC weapons) needs to be further examined and explored. This should include the recognition that certain competencies and skills may be transferable across the spectrum and between the two communities, for example, the NATO HFM Panel has recognised this, by merging Technical Groups on operational toxicology and medical aspects of CW-agents into one new group in 2007. What is apparent is that an appropriate organisation needs to be identified to champion EIHH management issues, and which bridges both communities. Informally, it has been suggested that this could be carried out by NATO Med NBC Working Group, but the feasibility of this would need to be explored further.

A key outcome, as discussed earlier, is the necessity to maintain contact and exchange knowledge and experience in this area. GBR has therefore tentatively agreed to host a 3rd International EIHH Workshop in late Summer / early Autumn 2007.

REPORT DOCUMENTATION PAGE

1. Recipient's Reference	2. Originator's References	3. Further Reference	4. Security Classification of Document
	RTO-TR-HFM-057 AC/323(HFM-057)TP/196	ISBN 978-92-837-0047-0	UNCLASSIFIED/ UNLIMITED

5. Originator	Research and Technology Organisation North Atlantic Treaty Organisation BP 25, F-92201 Neuilly-sur-Seine Cedex, France
6. Title	Biotechnologies for Assessment of Toxic Hazards in Operational Environments
7. Presented at/Sponsored by	Final Report of HFM-057/RTG-009.

8. Author(s)/Editor(s)	9. Date
Multiple	June 2008
10. Author's/Editor's Address	**11. Pages**
Multiple	154

12. Distribution Statement	There are no restrictions on the distribution of this document. Information about the availability of this and other RTO unclassified publications is given on the back cover.

13. Keywords/Descriptors

Aviation fuels	Hazards	Permethrins
Biological indicators	Health monitoring	Physical interactions
Biomarkers	Health risk communication	Sensitivity
Biomarkers of exposure	Inhalation hazards	Susceptibility
Biotechnology	JP8 and other petroleum products	Threat evaluation
Dermal exposure hazards	Models	Toxicology
Dosage	Neuropsychological measures	Vulnerability
Exposure	Neurotoxicological	

14. Abstract

Protection against toxicological threats that impair health and performance of military members requires identification of risks and methods to assess exposure. This group focused on markers of exposure for assessment of neurotoxicological threats from non-threat agents. Two model systems were examined in detail, permethrin and JP8. These represent relevant chemical mixtures that are inhalation and dermal exposure hazards with neurotoxicological potential. The group reviewed and reported research on approaches to assessing health and performance risks from these two models, ranging from neurobehavioral testing to special in vitro exposure test systems and cellular biomarkers. Interactions with physical factors (e.g., heat, dust, work/exercise), psychological stress, and other chemical exposures were evaluated. Research gaps in health risk communication strategies to mitigate risk and achieve optimal compliance with protective measures were also discussed. Two international Environmental and Industrial Health Hazard (EIHH) workshops paralleled the efforts of this panel and expanded contributions to this work. Further work in these areas is being conducted with agreements to continue sharing of information on approaches to assess neurotoxicological risks. Recommendations were made for further development of efficient processes for early predeployment consideration of potential threats, assessment and monitoring of neurochemical hazards, and lifecycle health monitoring of exposed individuals.

NORTH ATLANTIC TREATY ORGANISATION

RESEARCH AND TECHNOLOGY ORGANISATION

BP 25
F-92201 NEUILLY-SUR-SEINE CEDEX • FRANCE
Télécopie 0(1)55.61.22.99 • E-mail mailbox@rta.nato.int

DIFFUSION DES PUBLICATIONS
RTO NON CLASSIFIEES

Les publications de l'AGARD et de la RTO peuvent parfois être obtenues auprès des centres nationaux de distribution indiqués ci-dessous. Si vous souhaitez recevoir toutes les publications de la RTO, ou simplement celles qui concernent certains Panels, vous pouvez demander d'être inclus soit à titre personnel, soit au nom de votre organisation, sur la liste d'envoi.

Les publications de la RTO et de l'AGARD sont également en vente auprès des agences de vente indiquées ci-dessous.

Les demandes de documents RTO ou AGARD doivent comporter la dénomination « RTO » ou « AGARD » selon le cas, suivi du numéro de série. Des informations analogues, telles que le titre est la date de publication sont souhaitables.

Si vous souhaitez recevoir une notification électronique de la disponibilité des rapports de la RTO au fur et à mesure de leur publication, vous pouvez consulter notre site Web (www.rto.nato.int) et vous abonner à ce service.

CENTRES DE DIFFUSION NATIONAUX

ALLEMAGNE
Streitkräfteamt / Abteilung III
Fachinformationszentrum der Bundeswehr (FIZBw)
Gorch-Fock-Straße 7, D-53229 Bonn

BELGIQUE
Royal High Institute for Defence – KHID/IRSD/RHID
Management of Scientific & Technological Research for Defence, National RTO Coordinator
Royal Military Academy – Campus Renaissance
Renaissancelaan 30, 1000 Bruxelles

CANADA
DSIGRD2 – Bibliothécaire des ressources du savoir
R et D pour la défense Canada
Ministère de la Défense nationale
305, rue Rideau, 9e étage
Ottawa, Ontario K1A 0K2

DANEMARK
Danish Acquisition and Logistics Organization (DALO)
Lautrupbjerg 1-5, 2750 Ballerup

ESPAGNE
SDG TECEN / DGAM
C/ Arturo Soria 289
Madrid 28033

ETATS-UNIS
NASA Center for AeroSpace Information (CASI)
7115 Standard Drive
Hanover, MD 21076-1320

FRANCE
O.N.E.R.A. (ISP)
29, Avenue de la Division Leclerc
BP 72, 92322 Châtillon Cedex

GRECE (Correspondant)
Defence Industry & Research General Directorate, Research Directorate
Fakinos Base Camp, S.T.G. 1020
Holargos, Athens

HONGRIE
Department for Scientific Analysis
Institute of Military Technology
Ministry of Defence
P O Box 26
H-1525 Budapest

ISLANDE
Director of Aviation
c/o Flugrad
Reykjavik

ITALIE
General Secretariat of Defence and National Armaments Directorate
5th Department – Technological Research
Via XX Settembre 123
00187 Roma

LUXEMBOURG
Voir Belgique

NORVEGE
Norwegian Defence Research Establishment
Attn: Biblioteket
P.O. Box 25
NO-2007 Kjeller

PAYS-BAS
Royal Netherlands Military Academy Library
P.O. Box 90.002
4800 PA Breda

POLOGNE
Centralny Ośrodek Naukowej Informacji Wojskowej
Al. Jerozolimskie 97
00-909 Warszawa

PORTUGAL
Estado Maior da Força Aérea
SDFA – Centro de Documentação
Alfragide
P-2720 Amadora

REPUBLIQUE TCHEQUE
LOM PRAHA s. p.
o. z. VTÚLaPVO
Mladoboleslavská 944
PO Box 18
197 21 Praha 9

ROUMANIE
Romanian National Distribution Centre
Armaments Department
9-11, Drumul Taberei Street
Sector 6
061353, Bucharest

ROYAUME-UNI
Dstl Knowledge Services
Information Centre
Building 247
Dstl Porton Down
Salisbury
Wiltshire SP4 0JQ

SLOVENIE
Ministry of Defence
Central Registry for EU and NATO
Vojkova 55
1000 Ljubljana

TURQUIE
Milli Savunma Bakanlığı (MSB)
ARGE ve Teknoloji Dairesi Başkanlığı
06650 Bakanliklar
Ankara

AGENCES DE VENTE

NASA Center for AeroSpace Information (CASI)
7115 Standard Drive
Hanover, MD 21076-1320
ETATS-UNIS

The British Library Document Supply Centre
Boston Spa, Wetherby
West Yorkshire LS23 7BQ
ROYAUME-UNI

Canada Institute for Scientific and Technical Information (CISTI)
National Research Council Acquisitions
Montreal Road, Building M-55
Ottawa K1A 0S2, CANADA

Les demandes de documents RTO ou AGARD doivent comporter la dénomination « RTO » ou « AGARD » selon le cas, suivie du numéro de série (par exemple AGARD-AG-315). Des informations analogues, telles que le titre et la date de publication sont souhaitables. Des références bibliographiques complètes ainsi que des résumés des publications RTO et AGARD figurent dans les journaux suivants :

Scientific and Technical Aerospace Reports (STAR)
STAR peut être consulté en ligne au localisateur de ressources uniformes (URL) suivant: http://www.sti.nasa.gov/Pubs/star/Star.html
STAR est édité par CASI dans le cadre du programme NASA d'information scientifique et technique (STI)
STI Program Office, MS 157A
NASA Langley Research Center
Hampton, Virginia 23681-0001
ETATS-UNIS

Government Reports Announcements & Index (GRA&I)
publié par le National Technical Information Service
Springfield
Virginia 2216
ETATS-UNIS
(accessible également en mode interactif dans la base de données bibliographiques en ligne du NTIS, et sur CD-ROM)

NORTH ATLANTIC TREATY ORGANISATION

RESEARCH AND TECHNOLOGY ORGANISATION

BP 25

F-92201 NEUILLY-SUR-SEINE CEDEX • FRANCE

Télécopie 0(1)55.61.22.99 • E-mail mailbox@rta.nato.int

DISTRIBUTION OF UNCLASSIFIED RTO PUBLICATIONS

AGARD & RTO publications are sometimes available from the National Distribution Centres listed below. If you wish to receive all RTO reports, or just those relating to one or more specific RTO Panels, they may be willing to include you (or your Organisation) in their distribution.

RTO and AGARD reports may also be purchased from the Sales Agencies listed below.

Requests for RTO or AGARD documents should include the word 'RTO' or 'AGARD', as appropriate, followed by the serial number. Collateral information such as title and publication date is desirable.

If you wish to receive electronic notification of RTO reports as they are published, please visit our website (www.rto.nato.int) from where you can register for this service.

NATIONAL DISTRIBUTION CENTRES

BELGIUM
Royal High Institute for Defence – KHID/IRSD/RHID
Management of Scientific & Technological Research for Defence, National RTO Coordinator
Royal Military Academy – Campus Renaissance
Renaissancelaan 30
1000 Brussels

CANADA
DRDKIM2 – Knowledge Resources Librarian
Defence R&D Canada
Department of National Defence
305 Rideau Street, 9th Floor
Ottawa, Ontario K1A 0K2

CZECH REPUBLIC
LOM PRAHA s. p.
o. z. VTÚLaPVO
Mladoboleslavská 944
PO Box 18
197 21 Praha 9

DENMARK
Danish Acquisition and Logistics Organization (DALO)
Lautrupbjerg 1-5
2750 Ballerup

FRANCE
O.N.E.R.A. (ISP)
29, Avenue de la Division Leclerc
BP 72, 92322 Châtillon Cedex

GERMANY
Streitkräfteamt / Abteilung III
Fachinformationszentrum der Bundeswehr (FIZBw)
Gorch-Fock-Straße 7
D-53229 Bonn

GREECE (Point of Contact)
Defence Industry & Research General Directorate
Research Directorate, Fakinos Base Camp
S.T.G. 1020
Holargos, Athens

HUNGARY
Department for Scientific Analysis
Institute of Military Technology
Ministry of Defence
P O Box 26
H-1525 Budapest

ICELAND
Director of Aviation
c/o Flugrad, Reykjavik

ITALY
General Secretariat of Defence and National Armaments Directorate
5th Department – Technological Research
Via XX Settembre 123
00187 Roma

LUXEMBOURG
See Belgium

NETHERLANDS
Royal Netherlands Military Academy Library
P.O. Box 90.002
4800 PA Breda

NORWAY
Norwegian Defence Research Establishment
Attn: Biblioteket
P.O. Box 25
NO-2007 Kjeller

POLAND
Centralny Ośrodek Naukowej Informacji Wojskowej
Al. Jerozolimskie 97
00-909 Warszawa

PORTUGAL
Estado Maior da Força Aérea
SDFA – Centro de Documentação
Alfragide
P-2720 Amadora

ROMANIA
Romanian National Distribution Centre
Armaments Department
9-11, Drumul Taberei Street
Sector 6, 061353, Bucharest

SLOVENIA
Ministry of Defence
Central Registry for EU and NATO
Vojkova 55
1000 Ljubljana

SPAIN
SDG TECEN / DGAM
C/ Arturo Soria 289
Madrid 28033

TURKEY
Milli Savunma Bakanlığı (MSB)
ARGE ve Teknoloji Dairesi Başkanlığı
06650 Bakanliklar – Ankara

UNITED KINGDOM
Dstl Knowledge Services
Information Centre
Building 247
Dstl Porton Down
Salisbury, Wiltshire SP4 0JQ

UNITED STATES
NASA Center for AeroSpace Information (CASI)
7115 Standard Drive
Hanover, MD 21076-1320

SALES AGENCIES

NASA Center for AeroSpace Information (CASI)
7115 Standard Drive
Hanover, MD 21076-1320
UNITED STATES

The British Library Document Supply Centre
Boston Spa, Wetherby
West Yorkshire LS23 7BQ
UNITED KINGDOM

Canada Institute for Scientific and Technical Information (CISTI)
National Research Council Acquisitions
Montreal Road, Building M-55
Ottawa K1A 0S2, CANADA

Requests for RTO or AGARD documents should include the word 'RTO' or 'AGARD', as appropriate, followed by the serial number (for example AGARD-AG-315). Collateral information such as title and publication date is desirable. Full bibliographical references and abstracts of RTO and AGARD publications are given in the following journals:

Scientific and Technical Aerospace Reports (STAR)
STAR is available on-line at the following uniform resource locator: http://www.sti.nasa.gov/Pubs/star/Star.html
STAR is published by CASI for the NASA Scientific and Technical Information (STI) Program
STI Program Office, MS 157A
NASA Langley Research Center
Hampton, Virginia 23681-0001
UNITED STATES

Government Reports Announcements & Index (GRA&I)
published by the National Technical Information Service
Springfield
Virginia 2216
UNITED STATES
(also available online in the NTIS Bibliographic Database or on CD-ROM)

ISBN 978-92-837-0047-0